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Original article

Chemoprotective effect of vitexin against cisplatin-induced biochemical, spermatological, steroidogenic, hormonal, apoptotic and histopathological damages in the testes of Sprague-Dawley rats

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ABSTRACT

Cisplatin (CP) is one of the most widely used antineoplastic drugs, which possesses the potential to treat a variety of malignancies. However, it displays numerous side effects as well. Male reproductive dysfunction is one of the most adverse side effects of CP. Vitexin is a naturally occurring flavonoid, which exhibits remarkable antioxidant properties. Present study was designed to evaluate the protective effects of vitexin on CP-induced damages on testes. 48 Sprague-Dawley rats were equally distributed into 4 groups: control, cisplatin (CP), cisplatin + vitexin (CP + VIT) and vitexin (VIT). After 14 days of treatment, evaluation of biochemical, spermatogenic, steroidogenical, hormonal, apoptotic and histopathological parameters was carried out. CP damaged the biochemical profile by reducing activity of CAT, SOD, GPx and GSR, while level of MDA and ROS was increased. It also decreased sperm motility, viability, number of hypo-osmotic tail swelled spermatozoa and epididymal sperm count, besides increasing the sperm morphological anomalies. Moreover, levels of LH, FSH and plasma testosterone were reduced. CP reduced the gene expression of testicular anti-apoptotic marker (Bcl-2) and steroidogenic enzymes (3β-HSD, 17β-HSD and StAR), but upregulated the gene expressions of apoptotic markers (Bax and Caspase-3). Besides, CP led to histopathological damages in testicular tissues. However, vitexin reversed all aforementioned damages in testes. Therefore, it is concluded that vitexin could play an effective role as a therapeutic agent against CP-prompted testicular toxicity due to its antioxidant, anti-apoptotic and androgenic potential.

1. Introduction

Infertility is a cause of paramount stress in society since ages. According to some recent statistics, there are roughly 60–80 million couples worldwide suffering from infertility issues and about 50% of them are due to male partners (Kumar and Singh, 2015). Continuous advancements in chemotherapy have increased life expectancy up to 5 years in about 82% of cancer patients (Gatta et al., 2014). Regardless of this advancement, cancer convalescents face a plethora of testicular toxicities due to anticancer drugs. Therefore, protection of the reproductive potential of cancer survivors is a major concern of researchers these days (Gunnes et al., 2016; Haghi-Aminjan et al., 2018). Cisplatin (CP) is one of the chemotherapeutic drugs, which is extensively used to treat a variety of malignancies, for instance, testicular cancer (Smart et al., 2018) prostate cancer (Wei et al., 2015), head and neck cancer (Giralt et al., 2015), and bladder cancer (Zhu et al., 2015). However, it shows a number of side effects, i.e., hepatotoxicity (Ijaz et al., 2020a), ototoxicity, neurotoxicity (Ekinci-Akdemir et al.,

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2017), and mainly testicular toxicity (Ijaz et al., 2020b). CP treatment leads to the formation of DNA adducts and hinders the transcription and replication, which ultimately results in apoptosis. CP-prompted testicular toxicity is intense and irreversible (Al-Bader and Kilarkaje, 215)). A high proportion of polyunsaturated fatty acids (PUFAs) make testes extremely prone to CP-induced oxidative stress owing to excess production of reactive oxygen species (ROS) (Gevrek and Erdemir, 2018). Several CP-prompted damages in the male reproductive system, such as suppression of testicular steroidogenesis, germ cell apoptosis, and Leydig cell dysfunction, lead to oligospermia, azoospermia, and even partial or complete sterility in males (Heeba et al., 2016; Vassilakopoulou et al., 2016).

To cope with the oxidative stress prompted by CP in cancer patients, researchers have been looking for novel phytochemicals with antioxidant attributes since ages (Abdel-Daim et al., 2019). Vitexin (apigenin-8-C-D-glucopyranoside) is a polyphenolic group of phytochemicals, present in various herbs such as hawthorn herb and fenugreek (Kaviarasan et al., 2004). The dried leaves of hawthorn (*Crataegus pinnatifida*) were conventionally used as a traditional medicine in China (Wu et al., 2014). Vitexin displays prominent antioxidant (Nurdiana et al., 2017), anti-diabetic (Choo et al., 2012), anti-inflammatory (Venturini et al., 2018), neuroprotective (Chen et al., 2016) and cardioprotective (Che et al., 2016) properties. According to (He et al., 2016), vitexin may prove to be an effective therapeutic drug against multiple disorders due to its various pharmacological activities.

Therefore, the current study was planned to assess the ameliorative effect of vitexin on testicular and spermatological toxicity in CP-induced rats.

2. Materials and methods

2.1. Chemicals

Both CP and vitexin were procured from Sigma-Aldrich (Germany). The remaining chemicals were of analytical grade and procured from Sigma Aldrich. Enzyme-linked immunosorbent assay (ELISA) kits were obtained from BioCheck, Inc, USA for measuring the levels of FSH, LH and plasma testosterone.

2.2. Animals

Adult male Sprague-Dawley rats were kept in the bioterium of the University of Agriculture, Faisalabad, at 12 h/12 h light and dark cycle. The rats were adapted to laboratory conditions for a week, provided with water along with food pellets, and the temperature of the laboratory was maintained at 24–26 °C. Animals were handled according to guidelines for care and use of laboratory animals stated by the Institutional Ethics Committee, University of Agriculture, Faisalabad.

2.3. Experimental design

48 rats (weight 200 ± 10 g) were equally divided into 4 groups (n = 12 rats/group). The following treatments were provided to rats based on the group assigned: 1) Control group was injected 0.9% of saline intraperitoneally (i.p.) on day 1 and by oral gavage till 14 days, 2) CP group was injected 7 mg/kg b.wt. of CP (i.p.) on day 1, while saline was provided for 14 days by oral gavage, 3) CP + VIT group was injected a dose of CP (7 mg/kg b.wt. of CP on day 1), and 30 mg/kg b.wt. of vitexin was provided by oral gavage for 14 days, and 4) VIT group was also injected (i.p.) saline on day 1, while 30 mg/kg b.wt. of vitexin was given orally for 14 days. The dose of CP was chosen in accordance with Kaya et al. (2015) while the dose of VIT was selected according to a previous study by Sun et al. (2016). At the end of the experiment, rats were given anesthesia by diethyl ether and decapitation was performed. Plasma was separated by collecting the trunk blood in heparinized syringes. Blood was centrifugated for about 20 min at 322*g* and subsequently stored at −20 °C for further assessment. After dissecting the rats, a 10% formalin buffer was used to fix the left testicle for histomorphological assessment and the right testis was preserved at −80 °C to determine the activity of biochemical enzymes and for gene expression analysis. Tissues homogenization was conducted in PBS. Centrifugation was performed at 30,000 rpm for ten minutes. Ultimately, various assays were performed using supernatant.

2.4. Biochemical assessment

Activities of catalase (CAT) were evaluated by the procedure stated by (Chance and Maehly, 1955). Absorbance in mixture was determined at 240 nm and its results were shown as unit/mg protein. Superoxide dismutase (SOD) activities were determined by following the technique of Kakkar et al. (1984). Its enzymatic activites were evaluated by noticing the colour darkness (at 560 nm) and results were exhibited as unit/mg protein. Activities of glutathione peroxidase (GPx) were computed in accordance with the method reported by Lawrence and Burk (1976). Its results were also displayed as unit/mg protein. Activity of GSR was calculated at 25 °C by computing the NADPH disappearance at 340 nm by procedure of Carlberg and Mannervik (1975) and results were shown as nM NADPH oxidized/min/mg tissue. The method to estimate the malondialdehyde (MDA) level was described by Ohkawa et al. (1979). Analysis was performed by a spectrophotometer at 535 nm and the mean value was displayed as nmol/g tissue. The level of reactive oxygen species (ROS) was assessed by following the technique of Hayashi et al. (2007). At 505 nm, the absorbance was calculated and final results of ROS were stated as unit/mg tissues.

2.5. Evaluation of motility, viability, epididymal sperm count and structural abnormality of sperms

Spermatozoa from the caudal portion of epididymis were taken for estimation of motility. Sperm viability was evaluated by eosin/nigrosin staining, followed by a microscopic assessment. Hemocytometer was used for counting epididymal sperms (Yokoi et al., 2003). Additionally, the percentage of morphological abnormalities of head, tail and mid-piece of sperms was determined by following the procedure of (Cao et al., 2017).

2.6. Hypo-osmotic swelling test

The integrity of the sperm membrane was assessed by the hypo-osmotic swelling (HOS) test by following the procedure described by Correa and Zavos (1994). HOS test was carried out by placing 20 μL of semen in 180 μL of fructose solution keeping the osmotic pressure at 80 mOsm/L for about 20 min. After incubating and then mixing, the sperms were stained with eosin and nigrosin. Finally, 200 spermatozoa with swollen and non-swollen tails were counted using the light microscope (40× magnification).

2.7. RNA extraction and real-time quantitative polymerase chain reaction (qRT-PCR)

To determine gene expressions of 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) and steroidogenic acute regulatory protein (StAR), as well as apoptotic marker (Bax, Bcl-2 and Caspase-3) expressions, qRT-PCR was performed. The 95 mg of testicular tissues, frozen previously at –

80 °C, was taken and crushed using mortar and pestle. TRIzol reagent (Invitrogen, Paisley, UK) was used for the total RNA isolation. The purity of extracted RNA was assessed by the NanoDrop-2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 260 nm with A260/280 nm and A260/230 nm absorbance ratio. Complementary DNA was produced by the reverse transcription of total RNA. qRT-PCR was executed by means of SYBR Green kit (Thermo Scientific, USA). Estimate of apoptotic markers and steroidogenic enzymes' expression was taken by $2^{-\Delta\Delta CT}$ keeping β-actin as reference gene (Livak and Schmittgen, 2001). Primer sequences of β-actin and target genes are shown in Table 1.

2.8. Evaluation of LH, FSH and plasma testosterone

ELISA kits were used for the assessment of the levels of LH (Catalog# BC 1031), FSH (Catalog# BC 1029) and plasma testosterone (Catalog# BC 1115) as per instructions manual. Their final values were shown as ng/mL tissue.

2.9. Histopathological examination of testes

The testicular tissues were fixed for about 48 h in a 10% formaldehyde solution and embedded in wax blocks. Sections of equal thickness (5 μm) were sliced from paraffin wax blocks and staining was carried out using hematoxylin and eosin. Finally, these histopathological samples were observed via light microscopy. Leica LB microscope was used for taking photographs of specimens.

2.10. Statistical analysis

The final values were displayed as Mean ± SEM. Statistical difference among various groups was determined by the help of one-way of analysis of variance (ANOVA) and then Tukey's test was applied. $P < 0.05$ was the significance level.

3. Results

3.1. Biochemical alterations

CP significantly ($P < 0.001$) lowered the activity of CAT, SOD, GPx and GSR, while elevated the level of MDA and ROS in the CP group compared to the control group (Table 2). However, vitexin supplementation with CP remarkably increased the activities of CAT ($P < 0.01$), GPx ($P < 0.05$), GSR ($P < 0.01$) and SOD ($P < 0.001$), while substantially ($P < 0.001$) decreased the ROS and MDA levels in comparison to CP-administered group. Nevertheless, no significant difference was noticed among rats of the VIT group and the control group (Table 2).

Table 1
Primers sequences used for qRT-PCR.

Gene	Primers 5′ → 3′	Accession number	PCR products (bp)
3β-HSD	F: GCCACCCTTTAACTGCCACT R: CTGTGCTGCTCCACTAGTGT	NM_0010079	119
17β-HSD	F: TATCCAGGTGCTGACCCCTT R: CAAGGCAGCCACAGGTTTCA	NM_054007	103
StAR	F: AGCGTAGAGGTTCCACCTGT R: ATACTGAGCAGCCACGTGAG	NM_031558	123
Bax	F: GCACTAAAGTGCCCGAGCTG R: CCAGATGGTGAGTGAGGCAG	NM_017059.2	148
Bcl-2	F: ACTGAGTACCTGAACCGGCA R: CCCAGGTATGCACCCAGAGT	NM_016993.1	139
Caspase-3	F: GTACAGAGCTGGACTGCGGT R: TCAGCATGGCGCAAAGTGAC	NM_012922.2	137
β-actin	F: AGGAGATTACTGCCCTGGCT R: CATTTGCGGTGCACGATGGA	NM_031144	138

3.2. Motility, viability, epididymal sperm count and hypo-osmotic swelling test of sperms

CP significantly ($P < 0.001$) abated sperm motility, viability, epididymal sperm count and the number of HOS coil-tailed sperms, while morphological sperm anomalies (sperm head, mid-piece and tail) were increased in CP-intoxicated group compared to control group (Table 3). Conversely, vitexin significantly ($P < 0.001$) reversed all these sperm indices to a normal state in the co-treated (CP + VIT) group versus the CP group. Moreover, no significant difference was noticed among the VIT group and the control group (Table 3).

3.3. LH, FSH and plasma testosterone

CP significantly ($P < 0.001$) reduced the level of LH, FSH and plasma testosterone in CP-intoxicated rats versus the control group (Table 4). However, vitexin brought significant ($P < 0.001$) restoration in the level of all three hormones in the CP + VIT treated group versus the CP group. Additionally, no significant difference was observed between the VIT and control groups (Table 4).

3.4. Expression of steroidogenic enzymes

CP significantly ($P < 0.001$) reduced the expressions of 3β-HSD, 17β-HSD and StAR in the CP group versus the control group (Fig. 1). Nonetheless, the expression of these steroidogenic enzymes was significantly ($P < 0.001$) regained in CP + VIT treated group versus CP-intoxicated rats. Besides, no significant difference was noticed between VIT-treated and control groups (Fig. 1).

3.5. Expression of apoptotic markers

CP significantly ($P < 0.001$) upregulated the gene expressions of Bax and Caspase-3, while the Bcl-2 expression was down-regulated in the CP group compared to control group (Fig. 2). On the contrary, vitexin significantly ($P < 0.001$) reversed the expression of these apoptotic proteins in the CP + VIT group compared to CP-administered group. However, no significant difference was seen in the group treated with VIT with respect to the control group (Fig. 2).

3.6. Histological observation

The histopathological alterations due to CP and vitexin treatment are shown in Table 5 and Fig. 3. CP administration in rats significantly ($P < 0.001$) lowered the (seminiferous) tubular diameter and height, along with the tunica albugineal height. Besides, it scaled up the luminal diameter of tubules. CP also minimized the number of spermatogonia, primary and secondary spermatocytes, along with spermatids in CP group related to control group. On the other hand, supplementation of cytoprotectant, vitexin, significantly ($P < 0.001$) restored all these structural damages as well as germ cell count in testicles of CP + VIT group compared to CP group. Besides, no significant difference was noticed between the VIT-treated group and the control group.

Table 2
Effect of CP and vitexin on biochemical indices in rats.

Groups	Control	CP	CP + VIT	VIT
CAT (U/mg protein)	7.51 ± 0.21	4.96 ± 0.10$^{###}$	6.72 ± 0.17**	7.57 ± 0.30***
SOD (U/mg protein)	5.42 ± 0.14	2.51 ± 0.12$^{###}$	4.64 ± 0.16***	5.51 ± 0.24***
GPx (U/mg protein)	11.7 ± 0.05	7.07 ± 0.10$^{###}$	9.74 ± 0.17*	11.8 ± 0.31***
GSR (nM NADPH oxidized/min/mg tissue)	3.73 ± 0.13	1.51 ± 0.16$^{###}$	2.92 ± 0.09**	3.79 ± 0.21***
MDA (nmol/g tissue)	0.71 ± 0.07	1.86 ± 0.07$^{###}$	1.03 ± 0.04***	0.65 ± 0.12***
ROS (U/mg tissue)	1.19 ± 0.11	7.17 ± 0.27$^{###}$	2.17 ± 0.11***	1.03 ± 0.13***

Results are represented in Mean ± SEM (n = 12 rats/group). ### p < 0.001 compared with control group. ** p < 0.01 and *** p < 0.001 with respect to CP-treated group.

Table 3
Effect of CP and vitexin on sperm indices in rats.

Groups	Control	CP	CP + VIT	VIT
Sperm count (millions/mL)	25.0 ± 0.93	14.4 ± 0.35$^{###}$	20.9 ± 0.42***	26.1 ± 0.66***
Sperm motility (%)	61.1 ± 1.23	30.5 ± 1.01$^{###}$	53.3 ± 1.10***	62.2 ± 2.28***
Hypo-osmotic swelled sperm count (%)	55.6 ± 1.11	33.7 ± 1.31$^{###}$	49.4 ± 1.49***	59.9 ± 0.93***
Dead sperms (%)	15.7 ± 0.80	64.8 ± 1.80$^{###}$	25.4 ± 1.12***	16.2 ± 1.34***
Abnormal sperm head (%)	3.54 ± 0.17	8.75 ± 0.20$^{###}$	4.79 ± 0.09***	3.53 ± 0.17***
Abnormal sperm mid-piece (%)	0.74 ± 0.04	4.48 ± 0.20$^{###}$	1.04 ± 0.07***	0.73 ± 0.04***
Abnormal sperm tail (%)	3.07 ± 0.10	8.96 ± 0.11$^{###}$	4.26 ± 0.11***	3.17 ± 0.25***

Results are represented in Mean ± SEM (n = 12 rats/group). ### p < 0.001 compared to control group. ** p < 0.01 and *** p < 0.001 with respect to CP-treated group.

Table 4
Effect of CP and vitexin on hormonal levels in rats.

Groups	Control	CP	CP + VIT	VIT
LH (ng/mL)	2.37 ± 0.08	1.04 ± 0.13$^{###}$	2.06 ± 0.05***	2.39 ± 0.09***
FSH (ng/mL)	3.49 ± 0.08	1.64 ± 0.06$^{###}$	2.85 ± 0.11***	3.56 ± 0.15***
Plasma testosterone (ng/mL)	4.33 ± 0.16	2.06 ± 0.15$^{###}$	4.09 ± 0.06***	4.42 ± 0.15***

Results are represented in Mean ± SEM (n = 12 rats/group). ### p < 0.001 compared with control group. ** p < 0.01 and *** p < 0.001 with respect to CP-treated group.

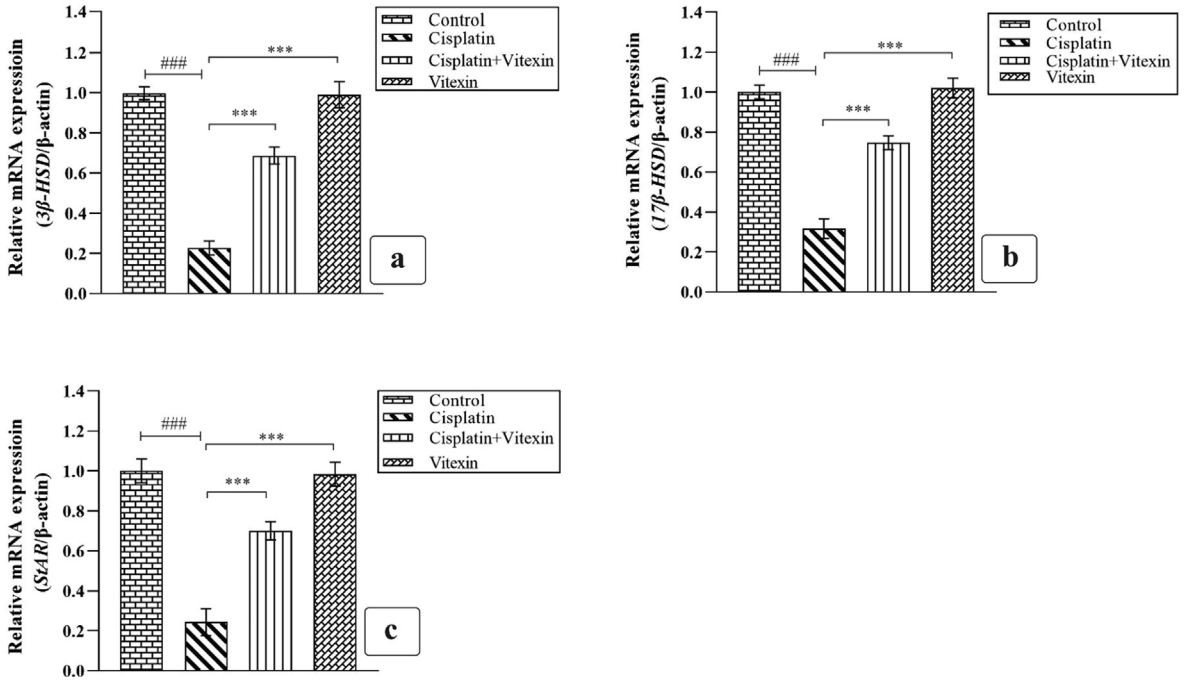

Fig. 1. Effect of CP and vitexin on the expressions of a) 3β-HSD, b) 17β-HSD, and c) StAR. All bars represent Mean ± SEM values (n = 12 rats/ group). ### p < 0.001 with respect to control group. ** p < 0.01 and *** p < 0.001 compared to CP-treated group.

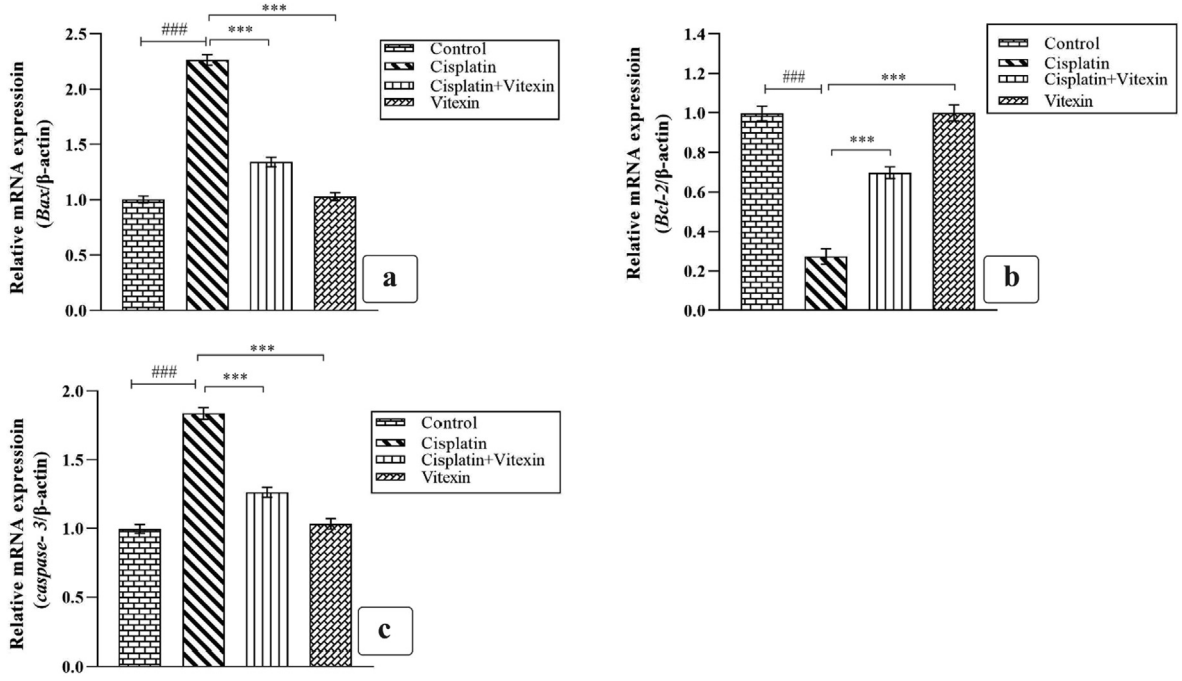

Fig. 2. Effect of CP and vitexin on the expressions of a) Bax, b) Bcl-2 and c) Caspase-3. All bars represent Mean ± SEM values (n = 12 rats/ group). ### p < 0.001 with respect to control group. ** p < 0.01 and *** p < 0.001 compared to CP-treated group.

Table 5
Effect of CP and vitexin on histomorphometry of rat testes.

Groups	Control	CP	CP + VIT	VIT
Tunica albuginea height (μm)	30.05 ± 1.82	13.61 ± 1.37$^{###}$	24.54 ± 0.63**	30.6 ± 1.37***
Diameter of tubules (μm)	229.5 ± 9.09	132.3 ± 5.54$^{###}$	208.66 ± 5.45***	237.5 ± 6.46***
epithelial height of tubules (μm)	87.59 ± 2.59	52.15 ± 2.07$^{###}$	73.07 ± 2.14**	86.66 ± 2.88***
Tubular lumen (μm)	58.22 ± 1.66	102.6 ± 1.87$^{###}$	73.29 ± 1.27***	57.41 ± 5.44***
Spermatogonia (n)	46.83 ± 0.89	23.90 ± 0.90$^{###}$	38.93 ± 0.34***	46.76 ± 0.70***
Primary spermatocyte (n)	33.64 ± 1.18	21.95 ± 1.16$^{###}$	29.88 ± 0.28**	33.94 ± 1.77***
Secondary spermatocyte (n)	28.61 ± 0.73	15.72 ± 0.55$^{###}$	24.43 ± 0.28***	29.32 ± 0.96***
Spermatids (n)	54.41 ± 0.61	26.30 ± 0.68$^{###}$	44.94 ± 1.28***	55.11 ± 1.68***

Results are represented in Mean ± SEM (n = 12 rats/group). ### p < 0.001 with respect to control group. ** p < 0.01 and *** p < 0.001 with respect to CP-treated group.

4. Discussion

CP is a chemotherapeutic drug that adversely affects the male reproductive system (Madhu et al., 2016). Administration of this drug initiates a cascade of damages due to oxidative stress and consequently results in infertility in the male cancer patients (Ahmed et al., 2011). CP administration causes excessive ROS generation, which results in an imbalance of antioxidant and pro-oxidant enzymes (Karimi et al., 2018) as well as leads to cell death in the healthy tissues apart from the tumor area (Dasari and Tchounwou, 2014). Furthermore, the over-generation of ROS culminates in the oxidation of macromolecules, protein and lipids, which indirectly affects semen quality (Spermon et al., 2006). According to Ghobadi et al. (2017), antioxidants usage holds several advantages in mitigating the side-effects of drugs apart from providing shielding effects to the male reproductive system during chemotherapy. Therefore, the current investigation aimed to investigate the alleviative role of vitexin on CP-generated testicular toxicity in rats by assessing biochemical profile, expression of steroidogenic and apoptotic enzymes, hormonal and spermatogenic alterations, as well as histopathologic state of testicular tissues.

Antioxidant defense system, including enzymes such as SOD, CAT and GPx, is imperative for the reduction of oxidative stress to avoid damage (Zia-Ul-Haq, 2021). CAT primarily transforms hydrogen peroxide to water (Aitken and Roman, 2008). SOD displays its antioxidant activities by transforming the highly-toxic superoxide ion into the less toxic component hydrogen peroxide and oxygen (El-Boshy et al., 2019). GPx acts to scavenge hydrogen peroxide (Ballatori et al., 2009). GSR protects the sulfhydryl groups as well as unsaturated fatty acids in spermatids against oxidation throughout the maturation phase of germ cells (Kaneko et al., 2002). In current study, CP elevated the degree of lipid peroxidation and caused oxidative stress, which was indicated by the increased level of ROS and MDA. Collectively, an increase in oxidative stress due to CP was evinced by the decrease in the activity of CAT, SOD, GPx and GSR, apart from elevation in level of MDA and ROS. However, vitexin remarkably increased CAT, SOD, GPx and GSR activities, along with the reduction in MDA and ROS, probably due to its antioxidant potential. According to Gao et al. (1999), the unique structure of vitexin with seven hydroxyls might be the reason behind its bioactivity, specifically theo-di-hydroxyl in A ring of flavonoids has been proved to play a part in their ROS scavenging potential.

An increase in ROS generation is one of the pivotal reasons behind male infertility. This ROS generation disrupts permeability of spermatozoon membrane, ultimately culminating in peroxidation of PUFAs (Bisht et al., 2017). Additionally, CP adversely affects

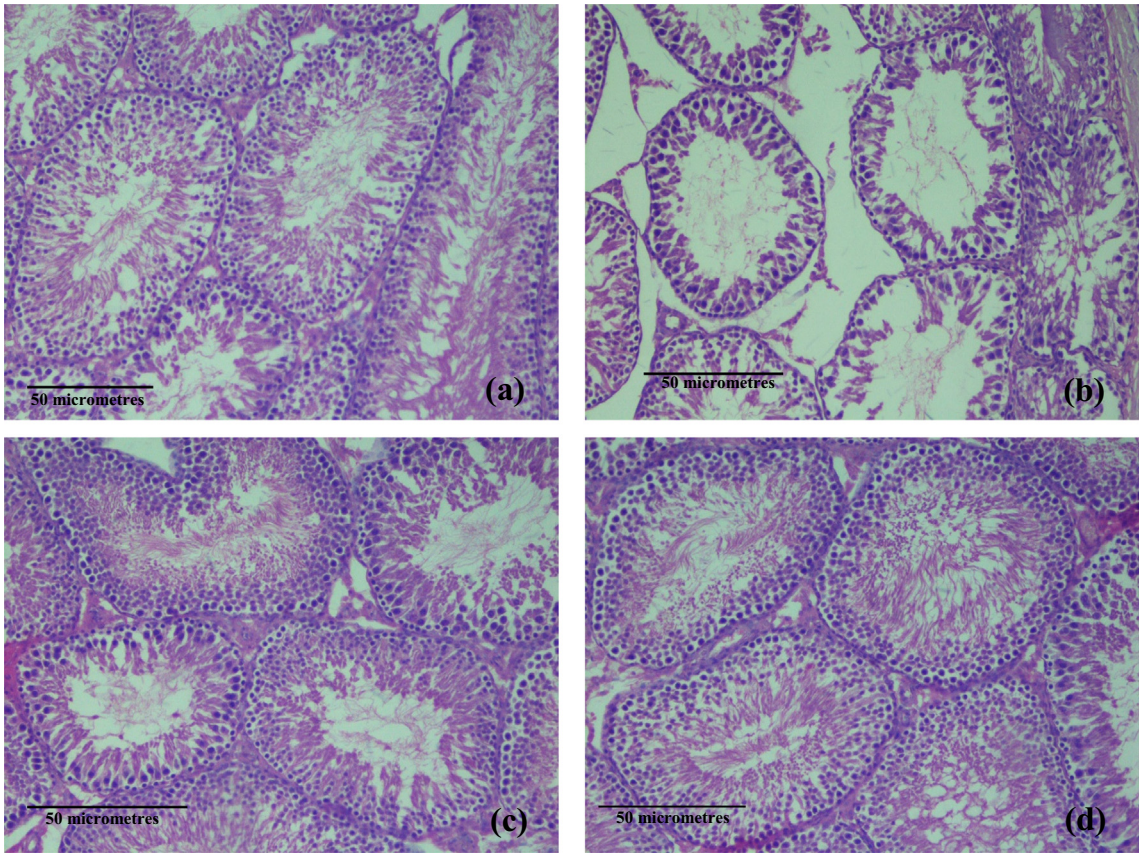

Fig. 3. Histopathological alterations by CP and vitexin in testicular tissues (H&E, 40x). (a): Control group exhibiting normal structure and lumen consisting of germ cells; (b): CP group exhibiting sloughed epithelium and lumen having less number of germ cells; (c): CP + VIT group displaying less sloughed epithelium and lumen containing higher number of germ cells in comparison to CP group; and (d): VIT group exhibiting compact epithelium and lumen full of germs cells as in control group.

the generation of ATP by damaging sperm mitochondria. This ATP reduction in spermatozoa harms flagellar activity, causing immobility of sperms (Ilbey et al., 2009). The disproportion of the oxidants and antioxidants and ROS-prompted membrane damage due to high levels of PUFAs in sperm might be the reason behind the distorted integrity of sperms, as confirmed by the HOS test. In current research, CP led to a profound reduction in epididymal sperm count, motility, and viability, along with the decrease in number of HOS coiled-tail sperms, while the higher level of abnormality was seen in the head, mid-piece and tail of sperms, which is in line with the previous literature (Elballat, 2016). But vitexin administration successfully resettled all the spermatogenic damages due to its potent ROS scavenging activity.

CP administration majorly lessened levels of all three hormones (LH, FSH, and plasma testosterone), which are necessary for spermatogenesis. LH accounts for the generation and maintenance of sperms by triggering testosterone production (Mantovani, 2007). Moreover, in combination with FSH, testosterone stimulates the growth of spermatids and the release of sperms (Chauhan et al., 2007). CP minimizes gonadotropins (LH and FSH) production along with testosterone, possibly due to disturbing the hypothalamus-pituitary–gonadal axis. In another investigation, it has been reported that CP increases ROS, which inhibits the activity of cytochrome cholesterol side-chain cleavage enzyme (P450scc), thereby lowering the testosterone level (García et al., 2012). On the contrary, vitexin improved the hormonal levels and the spermatogenic profile by stabilizing the functions of the hypothalamus-pituitary–gonadal axis, consequently enhancing the levels of gonadotropic hormones (LH and FSH) and testosterone, which ultimately restored spermatogenic damages.

To determine the mechanics behind the reduced level of testosterone after intoxication by CP, the expression of steroidogenic enzymes was assessed. According to (Ahmed et al., 2011) the underlying mechanism behind CP-induced testicular toxicity is the augmentation of the level of ROS as well as suppression of steroidogenesis. In the current study, CP significantly suppressed the expression of 3β-HSD, 17β-HSD and StAR. The key enzymes, 3β-HSD and 17β-HSD, regulate steroidogenic activities and play a primary role in androgenesis in the testicles (Aktas et al., 2012). Additionally, StAR is a rate-limiting enzyme that mediates cholesterol (testosterone precursor) transportation into the internal membrane of mitochondria to ensure testosterone biosynthesis (Liu et al., 2015; Ye et al., 2011). Lower expression of these androgenic enzymes due to CP-prompted oxidative stress resulted in the lower level of male reproductive hormone, testosterone. However, vitexin potentially increased testosterone levels by upregulating the expression of steroidogenic enzymes, probably by protecting mitochondria besides its antioxidant potential.

In the current study, CP escalated the expressions of Bax and Caspase-3, while lowered the Bcl-2 expression. These findings of CP-induced imbalance in apoptotic proteins were previously reported by previous investigations (Azab et al., 2020; Meligy et al., 2019). Bcl-2 and Bax are proteins, which are related to the Bcl-2 family. Bcl-2 encourages cells' survival by suppressing apoptosis, while Bax prompts cellular death (Gu et al., 2017). Reduction in Bcl-2 and elevation in Bax adversely alter the permeability of the mitochondrial membrane, resulting in a rise in the liberation of cytochrome *c* within the cytosol (Yang et al., 2017). This augmented cytochrome *c* in cytosol ultimately activates the expression of Caspase-3, which leads to apoptosis and cell death (Wang

et al., 2015). Nevertheless, vitexin mitigated these testicular damages via downregulating the expressions of Bax and Caspase-3 and increasing the Bcl-2 expression in rat testes. These results are in accordance with the findings of Wang et al. (2015) where vitexin has been reported to alleviate cerebral ischemia/reperfusion injury by augmentation in Bcl-2 expression and reduction in Bax expression in hippocampus and cortex.

According to previous studies, CP has consistently induced testicular-cell death due to the upsurge of ROS in the male reproductive system (Almeer and Abdel Moneim, 2018; Shati, 2019). In the present investigation, CP has elicited severe histoarchitectural damage in testicles by causing a decrease in (seminiferous) tubular height and diameter, as well as tunica albugineal height. Nevertheless, luminal diameter of tubules was increased. Moreover, CP scaled down the number of spermatogonia, primary and secondary spermatocytes, in addition to spermatids. CP-induced histopathological damages in testicular tissues (Amin et al., 2012) could be due to its adverse effect on the antioxidant defense system that created an imbalance among pro-oxidants and antioxidant enzymes. It was reported that CP gave rise to spermatogenic death and testicular damage due to the generation of oxidative stress (Ijaz et al., 2020b). However, vitexin elicited remarkable amelioration against the architectural degeneration of testicular tissues and the number of germ cells in the male reproductive system owing to its antioxidant and androgenic nature.

5. Conclusions

CP exposure prompted damages in spermatogenic, hormonal, and structural profiles in male albino rats. Additionally, the level of antioxidant enzymes as well as expression of apoptotic proteins and steroidogenic enzymes, exhibited a state of imbalance, thereby damaging the performance of the entire male reproductive system. However, vitexin restored all these damages in the aforementioned parameters owing to its antioxidant and androgenic potential. The current study could be very useful in treating the infertility issues in male cancer patients undergoing chemotherapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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