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Review

Effects of chemotherapeutic agents on male germ cells and possible ameliorating impact of antioxidants

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ABSTRACT

Treatment of cancer in young adults is associated with several side effects, particularly in the reproductive system. Detrimental effects of chemotherapy on the germ cells depend on many factors including primary semen parameters, the way of drug administration, the kind and dose of chemotherapeutic regimens, and the phase of spermatogenesis during the time of drug administration. Lack of appropriate fertility preservation treatments particularly in the affected children necessitates the introduction of methods to amend the harmful effects of chemotherapeutic agents on male germ cells. Several studies have assessed the toxic effects of chemotherapeutic agents in rodent models and tested a number of antioxidants to evaluate their possible impact on the preservation of sperm cells. In the present manuscript, we describe the effects of the mostly investigated chemotherapeutic drugs in this regard i.e., cisplatin, doxorubicin, paclitaxel, 5-fluorouracil, and cyclophosphamide. As several in vivo and in vitro studies have shown the impact of antioxidants on chemotherapy-induced damage of sperms, we also describe the protective effects of antioxidants in this regard.

Abbreviations: 8-Oxodg, 8-Oxodeoxyguanosine; ROS, Reactive oxygen species; O_2^-, Superoxide anion; H_2O_2, Hydrogen peroxide; •OH, Hydroxyl radicals; GSH, Reduced glutathione; TOS, Total oxidant status; TAS, Total antioxidant status; SOD, Superoxide dismutase; SOD1, Cu/Zn superoxide dismutase; SOD2, Mn superoxide dismutase; TLRs, Toll-like receptors; MAPKS, Mitogen-activated protein kinase; HMGB1, High mobility group box 1; MMP, Matrix metalloproteinase; Caspase, Cysteine-aspartic acid protease; DNMT3A, DNA methyltransferase 3A; DNMT3B, DNA methyltransferase 3β; LPO, Lipid peroxidation; MDA, Malondialdehyde; GSH-Px, Glutathione peroxidase; CAT, Catalase; ZRAB2, Zinc finger ran-binding domain-containing protein 2; ZFAN3, AN1-type zinc finger protein 3; LDH, Lactate dehydrogenase; G6PD, Glucose-6-phosphate dehydrogenase; SDH, Sorbitol dehydrogenase; ACP, Acid phosphatase; ALP, Alkaline phosphatase; TNF-α, Tumor necrosis factor alpha; IL-6, Interleukin 6; TGF-β1, Transforming growth factor beta 1; 3β-HSD, 3 Beta-hydroxysteroid dehydrogenase; 17β-HSD, 17 Beta-hydroxysteroid dehydrogenase; Nrf2, Nuclear factor (erythroid derived 2)-like 2; PHGPX, Phospholipid hydroperoxide glutathione peroxidase; TAP, Total antioxidant power; mTOR, Mammalian target of rapamycin; Cx43, Connexin 43; NO, Nitric oxide; CRP, C-reactive protein; LTB4, Leukotriene B4; T-AOC, Total antioxidant capacity; TNOS, Total nitric oxide synthases; LHCGR, Luteinizing hormone receptor; Scarb1, Lipoprotein-receptor; Cyp19a1, 19α-Hydroxylase; MT-1, Metallothionein-1; LH, Luteinizing hormone; FSH, Follicle-stimulating hormone; STAR, Steroidogenic acute regulatory protein; CYP11A1, P450 Side chain cleavage enzyme; CYP17A1, 17α-Hydroxylase; HO-1, Heme oxygenase-1; GR, Glutathione reductase; GSR, Testicular glutathione reductase; 4-HNE, 4-Hydroxynonena; IP, Intraperitoneal; D.C, Subcutaneous; IV, Intravenous; MPO, Myeloperoxidase; SF-1, Steroidogenic factor-1; HDAC2, Histone deacetylase 2; NQO-1, NADPH Quinone acceptor oxidoreductase 1; E2F1, E2F Transcription factor 1; HSPs, Heat shock proteins.

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1. Introduction

Cancer treatment strategies particularly chemotherapy can exert both temporary and perpetual harmful effects on male fertility [1]. These toxic effects of chemotherapeutic agents on the gonads depend on many factors including primary semen parameters, the route of drug administration, the type and dose of chemotherapeutic regimens, and the phase of spermatogenesis during the time of drug administration [1]. Toxic effects of chemotherapeutic agents on germ cells have also been detected in subjects exposed to these agents even before puberty. The observed oligozoospermia or azoospermia in these patients have been correlated with both the therapeutic regimen and the cumulative amount of administrated drug [2]. The hazard exerted on spermatogonial stem cells by cyclophosphamide and cisplatin has been verified [3,4]. The DNA damage induced by these agents can stimulate cell death cascades if not amended by cellular DNA repair mechanisms. Elevation of cleaved caspase-3 levels after treatment of spermatogonial stem cells with cyclophosphamide and cisplatin is indicative of activation of the apoptotic pathways. Moreover, chemotherapeutic agents have been found to induce apoptosis in spermatogonia and primary spermatocytes [5,6]. While apoptosis is the principal cell death route induced by cyclophosphamide, cisplatin, etoposide, and vincristine, some other chemotherapeutic agents particularly doxorubicin might induce testicular damage through induction of other routes such as necrosis or autophagy [7]. Although chemotherapeutic agents might also affect morphology or function of Sertoli cells, their impact on germ cells might indirectly result in cellular damage to the Sertoli cells [7]. Meanwhile, a number of antioxidants can protect gonads from these toxic effects. Most of these studies have been conducted in rodent models using the doses of chemotherapeutic agents that are administrated in human subjects. In the present manuscript, we describe the effects of cisplatin, doxorubicin, paclitaxel, 5-fluorouracil, and cyclophosphamide on male fertility and the protective effects of antioxidants in this regard. Fig. 1 depicts the side effects of chemotherapeutic drugs on testicular tissue via triggering cellular stress-mediated mitochondrial and endoplasmic reticulum-dependent apoptotic death in sperm cells which can eventually lead to infertility in men.

2. Cisplatin (CIS)

Cisplatin is a distinguished chemotherapeutic substance being administered for the management of various neoplastic conditions namely bladder cancer, head and neck cancers, lung cancer, and cancers originated from gonads. The effectiveness of cisplatin has been approved for carcinoma, germ cell tumors of testis, lymphoma, and sarcoma. This agent can make crosslinks with the purine bases on the DNA strand, thus blocking the DNA repair system, leading to DNA damage, and consequent induction of apoptotic pathways in cancer cells [8]. Cisplatin affects male reproductive potential through induction of cell apoptosis. It also decreases sperm parameters, induces abnormalities in hormones (FSH, LH, and testosterone) [9], and decreases the diameters of seminiferous tubules [10]. Also, cisplatin enhances the production of reactive oxygen species (ROS) that induce cell death [11]. In addition, cisplatin induces telomere dysfunction, thus might contribute to infertility through this mechanism [12]. In certain conditions, cisplatin can be hydrolyzed into differently charged reactive species which are dramatically more reactive compared with normal cisplatin and can inhibit mitochondrial respiration by interfering with oxidative phosphorylation. The subsequent affluence of Ca^{++} from the mitochondria would temporarily enhance the cellular Ca^{++} concentrations, disrupting normal Ca^{++} homeostasis [8]. The gonadotoxic effects of cisplatin have been assessed by several groups. Smart et al. have demonstrated induction of rapid and significant reduction in the germ cell population including stem germ cells in the prepubertal testis following treatment with cisplatin. This chemotherapeutic agent also reduced germ cell quantities even when being administrated at lower concentrations detected in patients' sera [13]. Yadav has reported a significant increase in lipid peroxidation and a decrease in reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in the testes of rats following treatment with cisplatin. He has also reported degenerative changes in several seminiferous tubules and germ cell depletion following such exposure, implying the harmful impact of this agent on testicular cells through induction of free radical-associated oxidative stress [14]. Notably, extracts of *H. sabdariffa* and *Z. officinale* have been shown to decrease the magnitude of cisplatin-associated harmful effects on spermatogenesis in rats. Both substances have returned the level of malondialdehyde (MDA) to the normal levels in the cisplatin-exposed samples and ameliorated the concentrations of SOD, GSH, and CAT [15]. Nilotinib as a multi tyrosine kinase inhibitor with antioxidant activity has also been shown to restore the testicular function in cisplatin-treated rats. Nilotinib has enhanced serum testosterone and sperm concentration and restored sperm survival. Meanwhile, Nilotinib has decreased levels of apoptotic markers namely JNKs and Caspase-3, and increased glutathione S-reductase (GSR), SOD, and total antioxidant capacity (TAC) levels while decreasing lipid peroxidation markers [16]. Table 1 demonstrates the summary of papers that assessed the impact of cisplatin/ antioxidants on male fertility. Fig. 2 illustrates the serious effects of reactive oxygen species triggered by cisplatin in semen which could cause a severe damage to germ cells as well as Leydig cells and bring testicular disorders and infertility in men.

3. Doxorubicin (DOX)

Doxorubicin is an anthracyline agent being administered for the management of malignancies originating from the breast, lung, stomach, thyroid, and ovary. Moreover, it is a therapeutic option for lymphoma, multiple myeloma, and sarcoma [49]. The anti-cancer effects of DOX are mediated through its intercalation with DNA and interruption of topoisomerase-II-associated DNA repair and production of free radicals and subsequent destruction of cell membranes, DNA, and protein molecules [50]. Moreover, DOX can induce transcriptional, miRNA, and DNA methylation alterations that disturb cascades participating in stress/apoptosis and survival and activity of germ cells leading to defects in this cell population and reproductive injury [51]. This chemotherapeutic agent affects male reproductive potential through induction of cell apoptosis as well as oxidative stress. It also decreases sperm parameters [52] and induces abnormalities in the levels of hormones (FSH, LH, and testosterone) [53]. Tremblay et al. have studied the effects of DOX in the induction of oxidative stress in rat spermatogonia and immature Sertoli cell lines. This agent has been shown to induce toxic effects on both cell lines in a time- and dose-dependent manner with the latter being more sensitive. Moreover, DOX could induce oxidative stress in the immature Sertoli cell line. As none of the four selected antioxidants (vitamin C, curcumin, carnitine, amifostine) could reduce the cytotoxic effects of DOX in Sertoli cells, authors have suggested that oxidative stress might not be the main cascade for DOX effects on these cells [54]. An experiment in rat testes has shown the deteriorative effects of DOX on testis histology, enhancement of apoptotic index, caspase-3 and HSP90 levels, and decrease in the serum testosterone concentrations. Yet, co-administration of thymoquinone has ameliorated the toxic effects of DOX in these animal models suggesting a protective effect for this substance against DOX-mediated testicular damage [55]. Besides, co-administration of vitamin C with DOX has been shown to decrease DOX-induced seminiferous epithelial injury in another rat model [56]. Another study has reported the beneficial effects of carnitine on the reduction of the late testicular and spermatic injuries resulted from the administration of DOX. Carnitine possibly exerts partial cytoprotection against the harmful effects of this chemotherapeutic agent in pre-pubertal rats [57]. Table 2 demonstrates the summary of papers that assessed the impact of DOX/ antioxidants on male fertility.

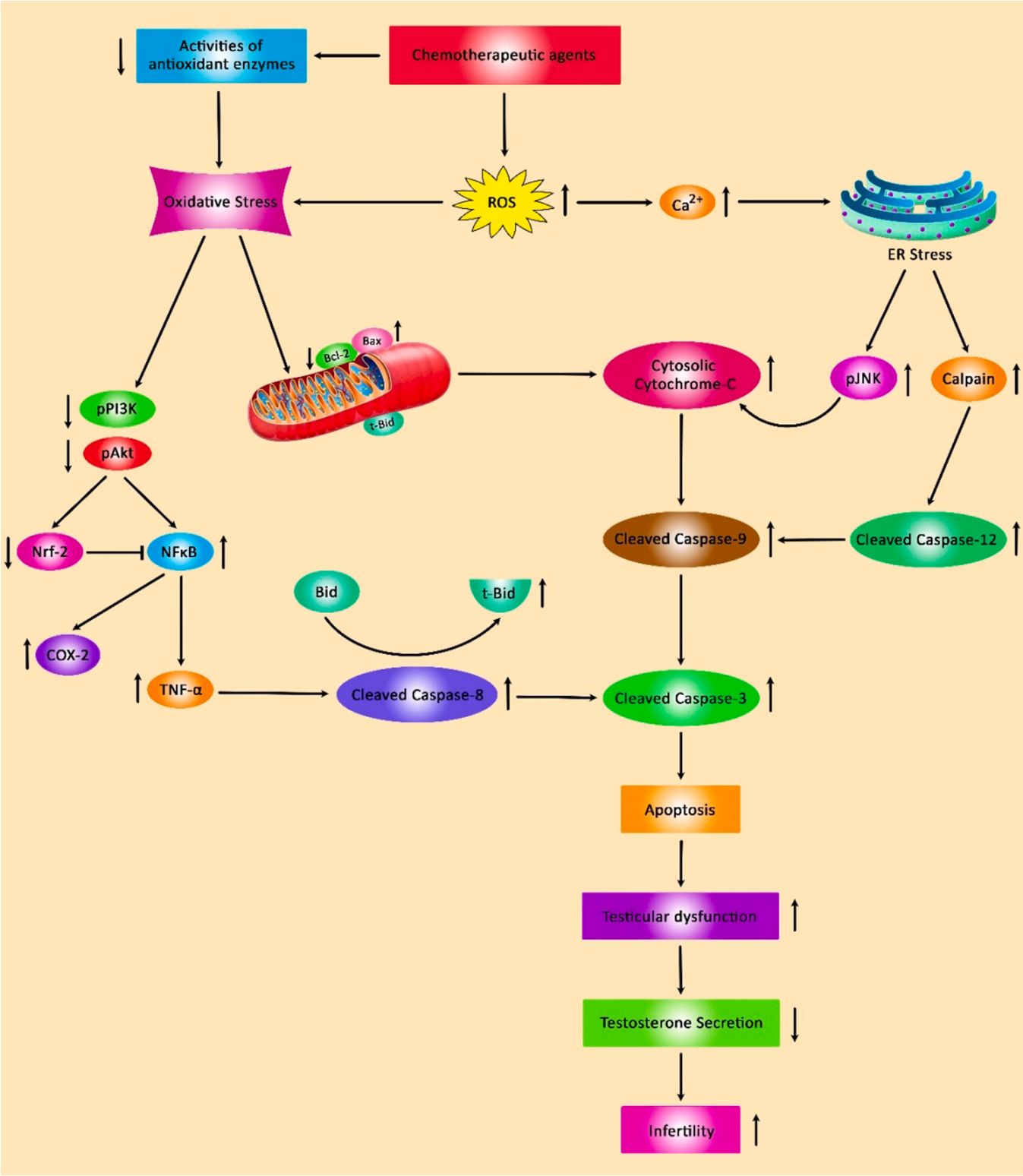

Fig. 1. The role of chemo drugs on testis tissue by triggering oxidative and ER stress-causing testicular dysfunction. Chemotherapy plays an effective role in exposing testes to cellular stress and causing testicular dysfunction as well as triggering stress-mediated apoptotic pathways in the testes. These agents could promote the expression levels of pro-inflammatory cytokines and translocation of NFκB into the nucleus and downregulate the expression of the transcription factor Nrf-2 to stimulate the oxidation. Besides, these drugs could suppress Bcl-2 expression, activate the expression of pro-apoptotic proteins (Bax, Bad, and Bid), increase intracellular Ca^{++} level, upregulate activities of caspase cascade and promote PARP cleavage. Accumulating evidence suggests that chemotherapy has an important part in mediating cellular stress-mediated mitochondrial and endoplasmic reticulum-dependent apoptotic death of the testicular cells which can finally cause a drop in testosterone levels as well as male infertility.

Table 1
Summary of papers that assessed the effects of cisplatin/antioxidants on male fertility.

Doses/duration, route administration	Antioxidant, doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
Cisplatin: 0.1, 0.5, 1.0 μg/ml 4 days (culture period) Doxorubicin: 0.05, 0.1, 0.5 μg/ml 4 days (culture period)	–	Mouse testicular cells	CC3 Mvh γH2AX	These agents could increase apoptosis in the germ cell population.	[13]
0.2 mg/kg daily for 30 days IP	–	Rat	VEGF HSP-70 8OHdG ICAM-1 VCAM-1	By altering 8-OHdG, VEGF, and HSP-70 expression could cause testicular cell death.	[17]
0.2, 0.3, or 0.4 mg/kg 5 days per week for 4 weeks IP	–	Rat	Clu GTPase Gimap4 Ptgds Tmeff1	Did not change the body, testis, or epididymis weights.	[18]
0.4 mg/kg, daily, for 60 days, IP	Curcumin: 20 mg/kg, daily, for 60 days, orally, starting 20 min prior to cisplatin, Vit C: 100 mg/kg, daily, for 60 days, orally, starting 20 min prior to cisplatin	Rat	–	An increase in the abnormal sperms was observed in the testes. Treatment with curcumin and vit. C could improve sperm quality.	[19]
1 mg/kg, daily, for 3 days IP	Acacetin (AC), 10, 25, 50 mg/kg, daily, for 3 and 10 days, IP	Mouse	MPO TNF-α	Decreases in sperm parameters, hormones (FSH, LH, and testosterone), and the diameters of seminiferous tubules were observed. AC could ameliorate the harmful effects of CIS on the testis.	[9]
1.1, 2,5, 5 mg/kg Single dose IP	Amifostine: 400 mg/kg daily for 7 days starting 24 h prior to cisplatin; IP Curcumin: 100 mg/kg daily for 7 days starting 24 h prior to cisplatin; orally Caffeic acid phenethyl ester (CAPE): 10 μmol/kg daily for 7 days starting 24 h prior to cisplatin; IP	Rat (post pubertal)	NF-kB/p65 Caspase-3 8-OHdG	An increase in expression of NF-kB/p65, Caspase-3, and 8-OHdG was observed in Leydig cells and germinal epithelium. Antioxidants (CAPE to a lesser extent) were able to counteract the toxicity induced by cisplatin.	[20]
1, 2 mg/kg Every other days for 16 days IP	–	Mouse	–	Could reduce the sperm count, the number of cells including spermatogonia, primary spermatocyte, and elongated spermatids, the thickness of germinal epithelial and seminiferous tubular diameter, and LH and testosterone levels.	[10]
1.5 mg/kg on days 1–5 of each week for 3 weeks IP	Zinc: 10 mg/kg, daily, for 9 weeks, orally	Rat	–	Treatment with zinc could ameliorate the adverse effects of CIS on sperm parameters, proper chromatin condensation, and testicular structure.	[21]
1.5 mg/kg on day 1–5 of three cycles (21 days) IP	–	Rat	SCARB1 StAR TOS CYP11A1 HSD17B6 CYP19A1 TAS	Induces degenerative changes in Leydig cells and inhibits the transcription of steroidogenic enzymes.	[22]
1.5 mg/kg on day 1–5 in each week for 9 weeks IP	Omega-3: 300 mg/kg, daily, for 9 weeks, orally	Rat	–	CIS induces DNA damage and influences sperm chromatin. Treatment with omega-3 could prevent these unfavorable changes.	[23]
1.5 mg/kg on days 1–5 of each week for 9 weeks IP	Omega-3: 300 mg/kg, daily, for 9 weeks, orally, an interval of 30 min for injections administered on the same day	Rat	–	During spermatogenesis, CIS affects chromatin integrity. Treatment with omega-3 could improve the cytotoxicity effect of CIS on sperm ubiquitination.	[24]
2.5 mg/kg single dose IP	–	Rat	–	CIS reduces testosterone, LH, and FSH, and induces damage in Sertoli cells, Leydig cells, and germ cells populations.	[25]
2.5 mg/kg daily for 5 days, 2 cycles IP	–	Mouse	–	CIS reduces the number of germ cells, the volume of the testes, and sperm parameters.	[26]
3 mg/kg daily for 3 days IP	Resveratrol: 20 mg/kg, daily, for 45 days, IP	Rat	ERK1/2 JNK Akt p53	Resveratrol by inhibiting ER stress, p53, ERK1/2, JNK, and activating AKT could protect against CIS-induced testicular damage and reproductive dysfunction.	[27]

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Table 1 (*continued*)

Doses/duration, route administration	Antioxidant, doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
			SOD CAT GPx GSH Caspase-3 calpain-1/ cleaved caspase-12		
5 mg/kg single dose IP	L-carnitine: 250 mg/kg, daily, for 3 days, IP, starting 1 h prior to cisplatin	Rat	–	CIS induces impaired sperm characteristics. L-carnitine could exhibit beneficial effects on these parameters.	[28]
5 mg/kg single dose IP	Eugenia Jambolana (EJE): 25 mg/kg, alternate day, for 7 days, IM; N-acetyl cysteine (NAC): 150 mg/kg, day 1 and 4, for 7 days, IP	Rat	3β-HSD TAC Caspase 3/8/9 SOD GST GR GSH GPx	Treatment with NAC or EJE could ameliorate the harmful effects of CIS on testicles.	[29]
5 mg/kg single dose IP	Grape Seed and Skin Extract (GSSE): 2.5 g/kg, daily, for 7 days, IP; Ulva rigida (U. rigida): 2.5 mg/kg, daily, for 7 days, IP	Rat	MDA SOD CAT	Treatment with GSSE or U. rigida extract could protect testicles against ROS formation induced by CIS.	[30]
5.5 mg/kg single dose IP	Tribulus terrestris (TT): 100, 300, 500 mg/kg, daily, for 4 days, IP	Mouse	NO ROS	The hydroalcoholic extract of TT by modulating oxidative stress via a mechanism linked to redox signaling could protect the testicles and sperm parameters against injury induced by CIS.	[31]
5.5 mg/kg single dose IP	TT: 100, 300, 500 mg/kg, daily, for 4 days, IP	Mouse	–	CIS reduces weights of the body and testes and enhances the apoptotic index in the testicle These parameters are improved when animals are treated with TT.	[32]
7 mg/kg single dose IP	Nilotinib: 20 mg/kg, daily for 10 days, orally, starting 24 h post to cisplatin	Rat	JNKs Caspase-3 GSR SOD TAC MDA 4-HNE	CIS increases apoptosis of spermatogenic cells and induces hyperplasia of Leydig cells. Treatment with nilotinib via inhibiting the JNK/ Caspase-3 pathway could ameliorate CIS-induced testicular injury.	[16]
7 mg/kg single dose IP	Tadalafil (TDF): 5 mg/kg, daily, for 6 days, orally; Zinc protoporphyrin-IX (ZnPP): 50 mmol/kg, daily, for 6 days, IP, Both of them starting 30 min prior to cisplatin	Rat	Nrf2/HO-1 TNF-α iNOS Caspase-3 Bax Bcl-2 MDA NO GSH CAT	TDF could prevent CIS -induced changes in the quality of sperm, serum testosterone level, apoptosis, and ROS in testicles.	[33]
7 mg/kg single dose IP	Tert-butylhydroquinone (tBHQ): 50 mg/kg, daily, for 14 days, orally	Rat	NF-kB TNF-α IL-1β/10 Bax, Bcl-2 Caspase-3 StAR CYP11A1 3β-HSD 17β-HSD Nrf2 SOD, CAT GPx, GR GSH, H_2O_2	Pre-treatment with tBHQ before administration of CIS by a mechanism involved in ROS, apoptosis, and inflammation could preserve testicular steroidogenesis and spermatogenesis.	[34]
7 mg/kg single dose IP	Rutin: 75 mg/kg, daily, for 13 days, orally, starting just after cisplatin administration	Rat	CAT SOD POD GR	CIS reduces the number of spermatogonia, spermatocytes, and spermatids cells, epithelial height, daily sperm production, and intra-testicular testosterone concentrations. Co-treatment with Rutin could ameliorate the adverse effects of CIS on these parameters.	[35]
7 mg/kg single dose IP	Quercetin: 50 mg kg, daily, for 10 days, orally	Rat	TAC TOS	Quercetin could partially reverse some of the CIS-related deleterious impacts on the testis.	[36]

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Table 1 (*continued*)

Doses/duration, route administration	Antioxidant, doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
			MDA CAT SOD XO		
7 mg/kg single dose IP	RES: 10 mg/kg, daily, for 5 days, orally	Rat	MDA GSH MPO	Treatment with resveratrol could reduce the number of apoptotic cells and prevent atrophic tubules and vacuole in germinal epithelial cell formation.	[37]
7 mg/kg single dose IP	GSPE: 200, 400 mg/kg, daily, for 15 days, orally, starting 10 days prior to cisplatin	Rat	Bax Caspase-3 Bcl-2	GSPE could protect testicular tissue against CIS by inhibiting the expression of apoptotic relative genes.	[38]
7 mg/kg single dose IP	Diospyros lotus (DL): 1000 mg/kg, daily, for 10 days, orally, starting just after cisplatin administration	Rat	SOD GPx CAT GSH	DL had protective effects against CIS-induced testicular damage and oxidative stress in rats. CIS altered sperm parameters and serum testosterone levels.	[39]
7 mg/kg single dose IP	β-glucan: 50 mg/kg, daily, for 14 days, orally	Rat	SOD CAT GPx GSH	Treatment with B-glucan could reduce CIS-induced oxidative and histopathological damage.	[40]
7 mg/kg single dose IP	Hemin: 40 mmol/kg, single dose, S.C, starting 30 min prior to cisplatin	Rat	MDA NO CAT GSH HO-1 Caspase-3 Bax/Bcl-2	Treatment with Hemin by upregulating the HO-1 could alleviate cisplatin-induced reproductive toxicity. Also, Hemin could induce cell death by increasing the Bax/ Bcl-2 ratio in PC3 cells.	[41]
7 mg/kg single dose IP	Maclura pomifera (MP): 500 mg/kg, daily, for 5 days, orally, starting 5 days before and continuing 5 days after the cisplatin administration	Rat	–	CIS could reduce some sperm parameters (motility and density) while increasing the rate of dead spermatozoa. Treatment with MP improved sperm survival rate.	[42]
7.5 mg/kg single dose	Amifostine: 400 mg/kg, single dose, IP, starting 30 min post to cisplatin;	Rat	MDA	CIS increases MDA levels and enhances the apoptotic rate in the germinal epithelium and Leydig cells.	[43]
IP	Melatonin, 5 mg/kg, daily, for 5 days, IP, starting 5 days before the cisplatin administration		GSH SOD Caspase-3	Melatonin and combinations (amifostine plus melatonin) could ameliorate the deleterious impacts of CIS by increasing GSH and SOD levels.	
10 mg/kg single dose	Grape Seed Procyanidins Extract (GSPE): 200, 100, 200, and 400 mg/kg, daily, for 15 days, orally.	Rat	OH	CIS induces the pathological changes of testicular tissue and decreases testosterone levels in serum	[44]
IP	Cisplatin administration occurred on the 11th day		CAT T-AOC SOD GSH LPO NO iNOS TNOS MT-1 HO-1 Lhcgr Scarb1 Star Cyp11a1 Cyp17a1 Cyp19a1 Hsd3b1 Hsd17b	Treatment with GSPE could improve these effects.	
10 mg/kg single dose IP	–	Rat	GSH MDA SOD CAT	CIS increases blood glucose level and glycosylated hemoglobin in the blood and also enhances the levels of MDA in testicular tissue	[14]
10 mg/kg single dose IP	Epigallocatechin-3-gallate (EG): 40, 80 mg/kg, day, for 5 days, IP, starting the same day of cisplatin administration	Rat	MDA TAS NO IL-6 IL-1β Cytochrome-C Bax Bcl-2 Caspase-3 TNF-α	EG protects testicular tissue against CIS by its antioxidant, anti-inflammatory, and antiapoptotic effects.	[45]
10 mg/kg single dose IP	Carvacrol (CARV): 75 mg/kg, daily, for 14 days, orally. cisplatin administration occurred on the 10th day	Rat	MDA GSH CAT GSH-Px SOD	CIS alters sperm parameters and induces testicular degeneration, and apoptosis, while treatment with CARV could reduce CIS-induced reproductive toxicity.	[46]
10 mg/kg single dose		Rat			[47]

(*continued on next page*)

Table 1 (*continued*)

Doses/duration, route administration	Antioxidant, doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
IP	Fraxinus Xanthoxyloides: 200, 400 mg/kg, daily for 45 days, orally; Silymarin: 100 mg/kg, daily, for 45 days, orally. Starting the same day of cisplatin administration		SOD, POD, CAT, GSR,	CIS decreases the FSH, LH, and plasma testosterone concentrations. F. xanthoxyloides extract could ameliorate the CIS-induced testicular toxicity.	
13 mg/kg single dose IP	Acetyl L-Carnitine: 200 mg/kg, single dose, S.C, starting 30 min prior to cisplatin	Rat	Caspase-3/8/9	CIS leads to weight loss and damage of the seminiferous tubules. Treatment with acetyl L-carnitine could protect against CIS-induced testicular damage.	[48]

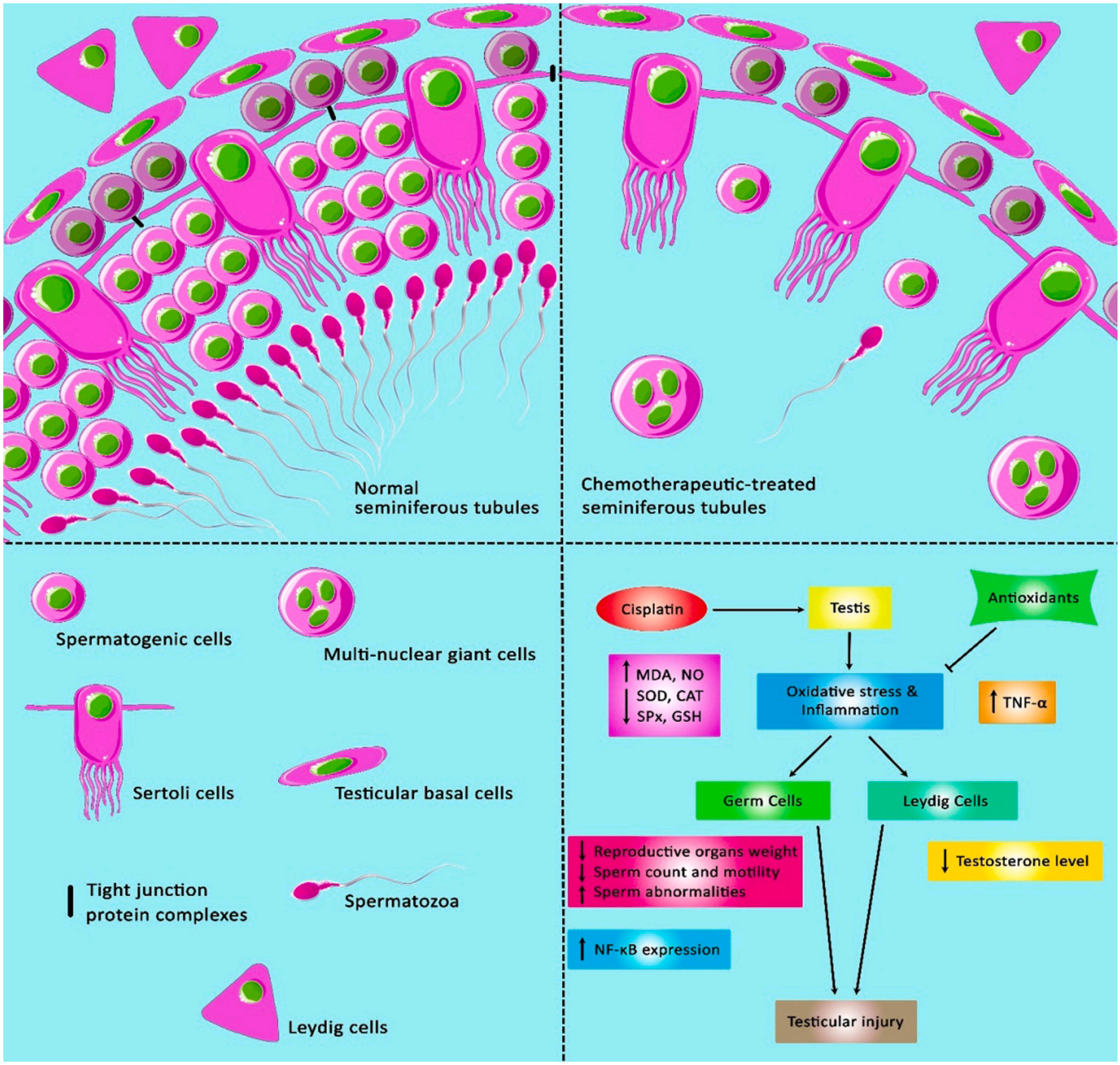

Fig. 2. Schematic illustration of cisplatin-induced testicular damage via activating the mechanism of oxidative stress in human semen. Cisplatin-based chemotherapy could be accompanied by activation of oxidative stress as well as inflammation in the testis's cells like germ cells and Leydig cells which could, in turn, lead to the creation of immature spermatozoa and male infertility. Antioxidant therapy could alleviate these side effects and play an effective role in maintaining a low level of oxidative stress in the semen, thereby allowing normal cell signaling cascades and spermatic function and preventing ROS-induced cell injury.

Table 2
Summary of papers that assessed the impact of DOX/antioxidants on male fertility.

Doses/duration, route of administration	Antioxidant, doses/duration, route of administration	Species	Targets/pathways	Observations	Ref.
0.001–10 μM 1–48 h	–	Spermatogonia (GC-6Spg), immature Sertoli (Ser-W3) cell lines	8-oxoDG ROS GSH	DOX induces oxidative stress in the Ser-W3 cell line	[54]
2 μM 24 h	Melatonin: 10 μM, for 24 h, the same time	Human pancreatic β-cell line (1.1B4)	HMGB1 TLR2 TLR4 MAPK NF-κB TAS TOS OSI SOD MMP Caspase-8	DOX activates caspase-8 via MAPKs/NF-κB pathway and enhances apoptosis. Treatment with Melatonin could reverse the depolarization of MMP and through decreasing TOS and increasing TAS could inhibit apoptosis.	[58]
0.2, 0.02 mg/kg twice a week for 5 weeks IP	–	Mouse	DNMT3a DNMT3b	DOX induces sparse abnormalities in testicular tissue	[52]
1.25, 2.5, 5 mg/kg weekly for 5 weeks IP	–	Rat	LPO GSH	DOX could increase the DNA damage of sperm and enhance abnormalities in the sperm head.	[59]
2 mg/kg once a week for 7 weeks IP	–	Rat	Caspase-3/8/9 MDA ZRAB2 ZFAN3	DOX could decrease some sperm parameters	[60]
2 mg/kg once a week for 8 weeks IP	–	Rat	MDA GSH-Px CAT SOD Caspase-8/3 NF-kB	DOX could induce activation of the intrinsic apoptotic pathway in testicles	[61]
3 mg/kg weekly for 3 weeks IP	–	Mouse	Bcl2 Caspase-3/6 Catsper1/3 Cyp17a1 Insl3 let-7 miRs-141-5p, 145, 190b, 767, 539-5p, 339-5p, 29b, 9-3p	DOX induces DNA methylation changes leading to germ cell loss and reproductive organ damage.	[51]
3 mg/kg 3 doses on 6th, 8th, and 10th days	Vit C: 0.5 mg/gr, daily, for 10 days, orally	Mouse	–	DOX could induce testicular damages in seminiferous tubules. Treatment with vit C reduced these effects.	[56]
3 mg/kg every 2 days for 3 doses IP	Ginkgo biloba extract 761 (EGb): 5 mg/kg, every 2 days for 3 doses, IP, EGb given 1 day before administration of DOX.	Rat	p53 Bcl-2 Caspase-3/8 MDA GSH GPx SOD1/2	DOX could reduce sperm production and lead to histological damages by increasing ROS and apoptosis. Pre-treatment with EGb could ameliorate the adverse effects of DOX.	[62]
3 mg/kg every other day for 3 doses IP	Taurine: 150 mg/kg, every other day for 3 doses, IP	Rat	p53 Fas Caspase-12 StAR SOD SDH ROS MAPKs MDA 17β-HSD 3β-HSD GPx GR GSH CAT JNK p38 MAPK	DOX by activating MAPKs and p53 via inducing DNA damage could stimulate testicular apoptosis. Taurine could attenuate these oxidative and apoptotic actions.	[63]
3 mg/kg on the 8th, 10th, 12th, 15th,	Zinc(Z): 10 mg/kg, daily, for 21 days, orally; Alogliptin (A): 20 mg/kg, daily, for	Rat	TGF-β1/NF-kB p65	DOX could decrease weights of the body weight and testicles, serum and testicular	[64]

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Table 2 (*continued*)

Doses/duration, route of administration	Antioxidant, doses/duration, route of administration	Species	Targets/pathways	Observations	Ref.
17th, and 19th days IP	21 days, orally, given 1 week before administration of DOX.		SOD GPx TNF-α IL-6 3β-HSD 17β-HSD	tissue zinc, and hormones (testosterone, LH, and FSH), but increase tissue inflammation factors and the percentage of dead and abnormal sperms. Zinc/alogliptin combination could reduce DOX-induced testicular toxicity.	
3 mg/kg once a week for 3 weeks IP	Ginseng intestinal metabolite-I (GIM-I): 50 mg/kg, daily, for 4 weeks, orally, given 2 weeks before administration of DOX.	Mouse	PHGPx	DOX could decrease the spermatogenic activities and serum levels of LDH and CPK. Treatment with GIM-I could result in improvement of parameters.	[65]
3 mg/kg at days 8, 10, 12, 15, 17, and 19 IP	Quercetin: 80 mg/kg, daily, for 21 days, orally; Sitagliptin: 10 mg/kg, daily, for 21 days, orally, …	Rat	GPx TAOC GSH AMP GLP-1	DOX could decrease the level of testosterone and LH, FSH, and GPx, increase the serum ALP while treating with quercetin, and sitagliptin could enhance the levels of testosterone, FSH, and LH, and decrease the level of ALP and LDH.	[53]
3 mg/kg on days 1, 4 and 7 IP	Atorvastatin (ATV): 5, 10, 20 mg/kg, daily, for 7 days, orally, 1 h before administration of DOX.	Mouse	MDA GSH	Treatment with ATV by decreasing oxidative stress and DNA damage had beneficial effects against DOX-induced toxicity.	[66]
3 mg/kg Every other day for 3 doses IP	Doxycycline: 2.5 mg/kg, every other day, for 3 doses, IP, (DcDox) given 1 day before administration of DOX.	Mouse	caspase-3 Bcl-2 Bcl-xL MDA SOD GPx	DOX could prevent DOX-induced upregulation of oxidative stress and suppress the pro-apoptotic effects of DOX by inhibiting the activity of apoptotic pathways.	[67]
3.7 mg/kg weekly for 47 days IV	Selenium (Se): 0.2 mg/kg, daily, for 47 days, orally; Thiocyanoacetamide (T): 10 mg/kg, daily, for 47 days, orally	Rat	–	DOX could reduce sperm quality and lead to a perturbation of ionic stability and a significant alteration of lipid metabolism. Both Se and T could improve sperm quality and partially restore spermatogenesis.	[68]
5 mg/kg single dose IP	–	Rat	MDA TAC	DOX could decrease the number of sperm and the body and testicular weights.	[69]
5 mg/kg twice a week for 2 weeks IP	Ellagic acid: 10 mg/kg, daily, for 14 days, orally; Rosmarinic acid: 75 mg/kg, daily, for 14 days, orally	Rat	TNF-α MDA GSH	DOX could increase the incidence of histopathological change in testicular tissue and decrease relative weight, testosterone levels, sperm parameters, and testicular glycogen. Treatment with ellagic and/or rosmarinic acid could reduce these effects.	[70]
5 mg/kg single dose IP	Carnitine: 250 mg/kg, single dose, IP	Rat	–	Carnitine could reduce the number of apoptotic germ cells, and also improve testis morphology and the quality of sperm in DOX-treated prepubertal rats.	[57]
6 mg/kg Daily for 3 days IP	Nano-zinc oxide (nZnO): 5 mg/kg, daily, for 3 days, IP, given 1 day before administration DOX.	Rat	TAP LPO	DOX could reduce plasma TAP, LPO, testosterone, and LH, some sperm parameters, and induce DNA damage, while treatment of nZnO could improve DOX-induced changes.	[71]
6 mg/kg 3 times weekly for 8 weeks IP	Zinc oxide nanoparticles (ZnO NPs): 3 mg/kg, five times weekly for 8 weeks, orally	Rat	MDA GSH CAT LPO	DOX could decrease the index weight of reproductive organs and some sperm parameters. Co-administration of ZnONPs could reduce the DOX-induced changes.	[72]
7.5 mg/kg single-dose IV	–	Rat	–	DOX could reduce testis weight and the number of pachytene spermatocytes.	[73]
7.5 mg/kg 1, 4, and 7th days P	Fluvastatin, 6 mg/kg, daily, for 7 days, orally, 24 h after administration of the last dose of DOX.	Rat	mTOR, SOD MMP-9 MDA LPO	Treatment with fluvastatin in DOX-induced testicular toxicity could prevent pathological damages.	[74]
10, 20, 40, 80, 320 μM 72 h	–	F9 testicular cell line	–	Treatment with DOX could reduce the cellular activity and ATP production 3–5 days later in the testicular cell line.	[75]
10 mg/kg single dose IP	Fullerenol (FLR) $C_{60}(OH)_{24}$, 100, 50 mg/kg, single dose, IP, 30 min before administration of Dox.	Rat	GST MDA LPO SOD GR GSSG GSH-Px	FLR $C_{60}(OH)_{24}$ could reduce DOX-induced oxidative stress.	[76]
10 mg/kg single dose IV	FLR $C_{60}(OH)_{24}$, 50, 100 mg/kg, single dose, IP, 30 min before administration of DOX.	Rat	TBA LPO ROS	DOX decreased the level of LPO. Treatment with FLR could prevent the appearance of DOX toxicity in testicles.	[77]
15 mg/kg once a week for 5 weeks IP	–	Rat	Nrf2 Caspase-3 Caspase-9	DOX could decrease antioxidant levels in the testicular milieu.	[78]

(*continued on next page*)

Table 2 (*continued*)

Doses/duration, route of administration	Antioxidant, doses/duration, route of administration	Species	Targets/pathways	Observations	Ref.
			Bcl-2 MDA Cytochrome-C GR		
15 mg/kg single dose IP	Thymoquinone (THQ): 10 mg/kg, daily, for 7 days	Rat	Caspase-3 HSP90 TAS TOS	THQ could reduce apoptosis, oxidative stress, and serum testosterone level, but increase some histopathological parameters such as MSTD.	[79]
15 mg/kg single dose IP	Naringenin (NG): 50 mg/kg, daily, for 10 days, orally, 6 days before administration of DOX.	Rat	Bcl-2 Bax Caspase-3 TNF-α IL-10 MDA TAS	DOX could decrease total antioxidant status, the serum testosterone and inhibin B, and testicular Bcl-2/Bax ratio. NG protects against these effects.	[80]
15 mg/kg single dose IP	Gallic acid (GA): 60, 120 mg/kg, daily, for 7 days, orally, concurrently with DOX.	Rat	Caspase-3 CAT SOD GSH NO GST	DOX by increasing oxidative stress in the testicular tissue could lead to severe toxicity in spermatogenesis and decrease the level of testosterone and some sperm parameters. Pre-treatment with GA had a protective role against the deleterious effect of DOX.	[81]
15 mg/kg daily for 10 days IP	Silymarin (SMN): 50 mg/kg, daily, for 7 days, orally, concurrently with DOX.	Rat	P53 E2F1 NO TAC	DOX decreased sperm quality values and induced oxidative as well as nitrosative stress and DNA fragmentation. Treatment with SMN could prevent these effects via the p53/E2F1 axis.	[82]
25 mg/kg 3 times a week for 2 weeks IP	Rutin: 50 mg/kg, daily, for 3 weeks, IP Ozone: 0.5–1 mg/kg, daily, for 3 weeks, rectal insufflations (RI)	Rat	MDA NO TAC	DOX could decrease sperm functions and testosterone levels, and enhance oxidative stress. Treatment with rutin and/or ozone could improve the mentioned parameters.	[83]
40 mg/kg single dose IP	Curcumin: 100, 200 mg/kg, daily, for 7 days, orally	Rat	MDA GSH GSH-Px CAT SOD	DOX could decrease some sperm parameters and cellular antioxidants. Treatment with curcumin could reduce these effects.	[84]

4. Paclitaxel (PTX)

PTX is an anticancer drug that polymerizes tubulin and converts it to stable microtubules. It directly attaches to microtubules, enhances their stability, inhibits their depolymerization by cold and calcium [85]. This agent could decrease the weight of all reproductive organs, affect sperm parameters, and testosterone level, and enhances the number of apoptotic germ cells and sperm DNA fragmentation [86]. It has two special properties i.e. having a specific binding site on the microtubule polymer and the capacity to polymerize tubulin without needing co-factors [85]. Chatzidarellis et al. have evaluated the effects of this kind of treatment in a number of male patients who have received taxane-based chemotherapy. They have demonstrated reduction of serum inhibin B and upsurge of serum FSH in all subjects following the accomplishment of the taxane-based chemotherapy. Moreover, they have reported reduction of testicular volume in a significant number of patients following completion of chemotherapy. Therefore, taxane-based chemotherapy has been associated with substantial gonadal injury in the primary phases after accomplishment of chemotherapy [87]. Sarıözkan et al. have investigated the potential protective impact of gilaburu (*Viburnum opulus L.*, Glb) fruit extract on docetaxel- and PTX-associated testis injury in rats. Both docetaxel and PTX have caused substantial reduction in absolute and relative weights of all reproductive organs, testosterone concentrations, sperm motility, and antioxidant capacity of the testis tissue. Notably, Glb ingestion has alleviated the PTX-induced reduction in absolute weights of epididimis, seminal vesicles, ventral prostate. Moreover, this fruit extract has amended the docetaxel- and PTX-associated defects in sperm parameters, disparities in oxidant/antioxidant coordination, increases in germ cell apoptosis and histological disturbances [88]. Table 3 demonstrates the summary of studies which assessed the effects of PTX/ antioxidants on male fertility.

5. 5-Fluorouracil

5-fluorouracil is an antimetabolite that induces testicular injury by epithelial sloughing and induction of cell death. An experiment in rat models has shown the effects of this agent on the induction of abnormalities in tubules and tubular atrophy. Notably, the exfoliated germ cells have been detected in the epididymis in a dose-dependent manner. Therefore, 5-fluorouracil causes tubular shrinkage and atrophy in rats [92]. Moreover, the 5-fluorouracil injection has resulted in a progressive reduction in the numbers of spermatocytes/spermatids within the seminiferous tubules of the mice. Such effects have been accompanied by apparent swelling and crazing of these tubules [93]. Another study in albino rats has shown the effects of 5-fluorouracil in the induction of sloughing of the seminiferous epithelium and the creation of giant cells [94]. This agent has also induced histopathological alterations including degeneration of seminiferous epithelium, decreased serum testosterone concentrations, and decreased weight of seminal vesicle and prostate without affecting LH or FSH levels or Leydig cells morphology [95]. In fact, 5-Fluorouracil can induce the formation of giant cells as a

Table 3
Summary of studies that assessed the effects of PTX/antioxidants on male fertility.

Doses/duration, route of administration	Antioxidant, doses/duration, route administration	Species	Targets/ pathways	Observations	Ref.
4 mg/kg weekly for 10 weeks IP	Cinnamon bark oil (CBO): 100 mg/kg, daily, for 10 weeks, orally, concurrently with PTX	Rat	MDA GSH CAT GSH-Px	PTX could decrease the weight of all reproductive organs, some sperm parameters, and testosterone level, as well as enhance the number of apoptotic germ cells and sperm DNA fragmentation. CBO treatment could reduce these effects.	[86]
4 mg/kg weekly for 10 weeks IP	Gilaburu (Glb): 100 mg/kg, daily, for 10 weeks, orally, concurrently with PTX	Rat	Bcl-2 Bax SOD GPx CAT MDA	PTX could decrease the weights of all reproductive organs, the motility of sperm, and levels of testosterone. Treatment with Glb could mitigate PTX-induced effects and improve the testicular histological and cytological damages.	[88]
5 mg/kg weekly for 4 weeks IP	Propolis: 50 mg/kg, daily, for 4 weeks, orally	Rat	8–OHdG MDA GSSH GSH	PTX could reduce some sperm parameters and increase the 8–OHdG and DNA damage. Treatment with propolis could improve the semen quality and protect testicles against toxicity induced by PTX.	[89]
7.5 mg/kg weekly for 4 weeks IP	Royal jelly (RJ), 0, 50, 100, 150 mg/kg, daily, for 4 weeks, orally	Rat	MDA TBA TTM NO E2f1	RJ treatment could lower the PTX-induced MDA and NO levels and improve the reduced sperm viability.	[90]
7.6 mg/kg single-dose IV	–	Rat	–	PTX could induce structural and functional disorders in gonocytes of all progenesis stages.	[91]

histopathological alteration and reduction of serum testosterone concentration as a biochemical alteration. Table 4 demonstrates the summary of studies that assessed the effects of 5-Fluorouracil on male fertility.

6. Cyclophosphamide (CP)

Cyclophosphamide is an alkylating agent. Alkylating agents are shown to exert the most gonadotoxic effects among other substances with the extent of gonadal injury being correlated with the dose of alkylating agent administrated [100]. Cyclophosphamide has been shown to decrease testicular weight, reduce sperm parameters (viability, motility, and count), and increase sperm abnormality (head, neck, and tail). Moreover, it affects levels of sex hormones [101]. Experiments in Wistar rats have shown a decrease in testes' weight and pathological changes such as fibrosis in this organ following treatment with cyclophosphamide [102]. Cyclophosphamide has also detrimental effects on the male reproductive system even in the prenatal stage. This drug has decreased body and testicular weight as well as sperm count, viability, and motility. Moreover, prenatal exposure to cyclophosphamide has increased abnormalities in the sperm head, neck, and tail [103]. Another experiment in Albino mice has shown harmful effects of cyclophosphamide sperm count, motility, and morphology and its effects on the induction of DNA damage in spermatozoa. This agent has also reduced inhibin B and testosterone levels and increased FSH levels. Notably, administration of ethanolic extract of *Moringa oleifera* leaves before chemotherapy has amended sperm functional features, reduced FSH, and surged inhibin B concentrations. Moreover, cyclophosphamide has decreased expression of Abp, while increased Transferrin, Fshr, and Gata4 levels in the testes. Administration *Moringa oleifera* leaves extract has ameliorated cyclophosphamide-induced injury through modulation of expression of genes contributing to the function of Sertoli and spermatogenic cells [104]. Administration of *Moringa oleifera* leaves extract before cyclophosphamide treatment has enhanced the level of SOD and CAT and decreased lipid peroxidation in the testis [105]. Table 5 demonstrates the summary of studies that assessed the effects of cyclophosphamide/ antioxidants on male fertility.

7. Human studies

Although most of the studies have assessed the effects of anti-cancer drugs on fertility parameters in animals, several studies have appraised such effects in human subjects. Most of these studies have appraised the effects of combinatorial regimens on male fertility, thus it is not possible to find the effects of individual agents. For instance, Meistrich et al. have analyzed semen parameters in patients with Ewing sarcoma and other types of sarcomas prior to, during, and following treatment with two chemotherapeutic regimens (combination of cyclophosphamide, DOX, and dacarbazine with or without vincristine). Although semen parameters have been comparable with controls prior to chemotherapy, azoospermia has appeared within 4 months of therapy. However, sperm production resumed in a number of cases after treatment. Normal sperm parameters have been detected in 40% of cases by 5 years following the accomplishment of chemotherapy. A small number of patients exhibited constant regaining of sperm production after that time. Notably, the cumulative dose of cyclophosphamide has been the furthermost important determining factor of regaining normospermic levels [150]. Administration of the chemotherapeutic regimen consisting of cyclophosphamide, DOX, vincristine, prednisone, and bleomycin in lymphoma has also been associated with induction of azoospermia in all treated patients. However, after the accomplishment of treatment, the proportion of patients with sperm recovery has enhanced increasingly over 5 years and plateaued by 7 years. At this time point, two-thirds of patients achieved normospermia. Notably, scattered gonadal radiation dose and cumulative cyclophosphamide dose have been the most important factors in regaining normal sperm count. On the other hand, administration of further drugs and IFN-α has not affected regaining normal sperm count [151]. In a small cohort of patients, Buchanan et al. have assessed sperm parameters of patients treated with cyclophosphamide. They have shown a return of spermatogenesis in less than half of patients within 15–49 months after completion of chemotherapy. They have reported no significant association between regaining of spermatogenesis and factors such as age, duration of chemotherapy or overall dose [152]. Moreover, in vitro studies on human sperms have shown induction of DNA damage by chemotherapeutic agents such as DOX [153]. Treatment with cyclophosphamide has been linked with testicular injury in a dose- and time-dependent mode. Independent studies have indicated the effects of this agent in diminution of the germ

Table 4
Summary of studies that assessed the effects of 5-Fluorouracil on male fertility.

Doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
5 mg/kg single dose IP	Mouse	–	5-FU could decrease the numbers of spermatocytes/ spermatids.	[93]
10, 50, and 100 mg/kg single dose IP	Rat	–	5-FU could induce seminiferous tubular atrophy and decrease testicle weight and the height of epithelium.	[92]
5, 10, and 15 mg/kg five consecutive days IP	Mouse	–	After 35 days, all doses increased the numbers of abnormal sperm and multinucleated giant cells.	[96]
10, 20, and 30 mg/kg five consecutive days IP	Rat	–	5-FU could decrease the sperm count in a dose- and time-dependent manner.	[97]
10, 50, and 100 mg/kg single dose IP	Rat	–	All doses of 5-FU could induce the formation of giant cells.	[94]
20 mg/kg, every other day for 1 month IP	Rat	VEGF, HSP-70, 8-OHdG, ICAM-1, VCAM-1	5-FU by enhancing conjugation among spermatogenic cells could form multinucleated giant cells with different structural patterns. 5-FU could also reduce VEGF, ICAM-1, and VCAM-1, while increasing DNA damage, 8-OHdG, and HSP-70.	[98]
20 and 30 mg/kg orally for 2-week or 4-week term	Rat	–	5-FU could lead to Sertoli cell vacuolation, decrease serum levels of testosterone, activin A, prolactin, and inhibin B, and increase seminiferous tubule degeneration.	[95]
25 mg/kg three consecutive days IP	Mouse	–	After 2 weeks, lack of spermatozoa, vacuolar degeneration in spermatocytes, and congestion of blood vessels in the interstitium were detected.	[99]

cell populations, leading to Sertoli cell-only tubule appearance, induction of fibrosis in interstitial spaces, and thickening of the basement membranes [154–156]. Administration of high dose of cyclophosphamide has been associated with extensive testicular injury with Sertoli cell-only tubules existing until 9 years following treatment [157,158]. Table 6 shows the results of human studies that appraised the effects of anti-cancer treatments on male fertility.

8. Discussion

Chemotherapeutic agents have detrimental effects on spermatogenesis processes. Administration of these drugs for childhood cancers can result in fertility problems considering the lack of fertility preservation treatments in this group. The difference in germ cell stages and levels of sex hormones between prepubertal and pubertal testicular tissues necessitates the evaluation of the effects of these drugs in each kind of sample individually. Numerous investigations have evaluated the effect of these drugs on prepubertal mouse/rat testis exposing testicular sections to clinically relevant doses of these agents. These studies have confirmed the induction of apoptosis in germ cells following exposure to these drugs. However, the development of sperm cells substantially differs between rodent and primate species, thus it is not possible to translate these results into a human. The establishment of primate models for the assessment of the effects of chemotherapeutic agents on male fertility has its own limitations. Notably, some studies have suggested specific effects of these drugs on germ cells with no harmful impact on somatic cells, i.e. Sertoli and interstitial cells. However, a number of studies have reported detrimental effects of chemotherapeutic agents on Sertoli and Leydig cells. Differences in the spermatogenesis stage and animal models might explain the discrepancy between the results of these studies.

Few human studies in this field have shown detrimental effects of chemotherapeutic agents on sex hormones, testis morphology, and semen parameters. However, these studies have not evaluated the mechanistic effects of these agents in human subjects. Animal studies have identified some targets and signaling pathways that are involved in these effects. In addition to the direct effects of chemotherapeutic agents on the induction of cell apoptosis, they can affect the oxidative stress responses in this tissue. Therefore, antioxidants might ameliorate the toxic effects of these agents in the testicular tissue. Among the assessed antioxidants, extracts of *H. sabdariffa* and *Z. officinale* have enhanced the activity of antioxidant enzymes and reinstated sperm motility in animal models treated with cisplatin [15]. Nilotinib, Amifostine, Melatonin, Curcumin, and vitamin C are among other substances with useful effects in this regard. The molecular cascades contributing to the preservative effects of these substances are largely unknown. However, some of these agents have multifaceted impacts. For instance, nilotinib can simultaneously increase antioxidant capacity, inhibit apoptotic cascades, and reestablish the regenerative aptitude of testes [16]. Several other herbal substances such as Olive Leaf Extract, Ginger, Garlic acid, Curcumin, Ceratonia Extract, Nerolidol, Aquilaria malaccensis, and Squid ink polysaccharide have also been shown to amend the detrimental effects of cyclophosphamide on male fertility. However, the underlying mechanism of their effects has not been clarified completely.

Induction of cell apoptosis and DNA fragmentation and production of ROS have shared mechanisms between antineoplastic drugs. Therefore, almost all of these drugs have been shown to affect the weight of the testis, diameter of seminiferous tubules, sperm parameters, and levels of sex hormones. The effects of CIS, PTX, CP, 5-Fluorouracil, and DOX on decreasing testicular weight have been documented by several studies. These agents also induce degenerative changes in seminiferous tubules and germ cell depletion along with changes in the histological structure of the seminiferous tubule. Semen volume, sperm concentration, sperm motility, sperm viability, and motility are semen parameters that are affected by chemotherapeutic agents. Moreover, chemotherapeutic agents can reduce testosterone, LH, and FSH levels, and induce damage in Sertoli cells, Leydig cells, and germ cells populations. However, a number of human studies have reported that such effects are transient with the furthermost important determining factor for regaining normal levels being the cumulative dose of the agent.

Moreover, cisplatin is used in co-administration with etoposide and bleomycin in several schedules. The administration of these drugs has been associated with cell damage, oxidative stress, metabolic disruption, and immune deficiency [168]. Therefore, antioxidants that modulate the effects of these agents on male fertility have clinical significance [169]. For instance, administration of Zinc supplements after chemotherapy with bleomycin has been shown to recover chromatin integrity, testicular organization, and spermatogenesis [170]. Besides, administration of Zinc after chemotherapy of testicular cancer with bleomycin has been demonstrated to reduce off-target consequences related to oxidative stress [21]. Antioxidants have also amended the detrimental effects of bleomycin, etoposide, and cisplatin on testicular function, pituitary-testicular axis, and fertility [171]. Additionally, melatonin has protective effects against bleomycin, etoposide, and cisplatin-associated toxic effects through moderating nitro-oxidative stress and apoptosis

Table 5
Summary of studies that assessed the effects of cyclophosphamide (CP)/antioxidants on male fertility.

Doses/duration, route administration	Antioxidant, doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
1.6 mg/kg single dose IP	Vit C: 0.88 mg/kg single dose, orally, 1 h interval	Rat	–	Vit C could improve sperm parameters in CP-induced reproductive toxicity.	[106]
5 mg/kg daily for 28 days orally	Ceratonia Extract: 300 mg/kg, daily for 28 days, orally, 4 h after and before CP induction.	Rat	MDA TAC LH FSH	Ceratonia extract could increase some sperm parameters, serum testosterone levels, and both serum and tissue total antioxidants against CP-induced reproductive toxicity.	[107]
5 mg/kg 3 days per week for 4 weeks orally	Alpha-Lipoic acid (LA) and Royal Jelly (RJ); 25 mg/kg and 1 g/kg, 3 times a week for 4 weeks, orally	Rat	MDA SOD	CP could lead to germ cell apoptosis and maturation arrest. Also, pyknosis and cytoplasmic vacuolation were observed in Sertoli cells. Administration of LA and RJ could improve the histopathology and ultrastructure of the testis.	[108]
5 mg/kg per day 4 weeks orally	Spirulina platensis (SP): 500 and 1000 mg/kg per day for 4 weeks, orally; Vit. E, 100 mg/ kg, daily for 4 weeks, IP. 4 h after CP induction.	Rat	MDA GPx	Co-administration with SP and vit E could improve histomorphometric alternations, the body and testes weights, and sperm count against CP-induced reproductive toxicity.	[109]
5 mg/kg daily for 28 days orally	Vit E: 200 mg/kg, daily for 28 days, orally	Rat	–	Vit E decreased vacuolated cytoplasm and pyknotic nuclei in the germinal epithelium and abnormal mitochondrial sheaths in the middle piece of sperm.	[110]
5 mg/kg daily for 28 days IP	–	Rat	–	CP led to the formation of many distorted shrunken seminiferous tubules, affected axoneme, fibrous sheath, and mitochondrial sheath of sperm, and increased dilated SER and mitochondria in the cytoplasm of Leydig cells.	[111]
6 mg/kg daily for 21 days orally	N-acetylcysteine: 100 mg/kg, daily for 21 days, orally	Rat	Bax Bcl-2	N-acetylcysteine could increase sperm count.	[112]
6.1 mg/kg daily for 50 days IP	American Ginseng: 500 mg/kg, daily for 50 days, orally, 1 h before CP induction.	Rat	MDA	Co-administration of American ginseng could increase testosterone levels and improve histologic changes induced by CP.	[113]
10 mg/kg single dose IP	–	Mouse	–	CP decreased the body and testicular weights, reduced sperm parameters (viability, motility, and count), and increased sperm abnormality (head, neck, and tail).	[103]
10 mg/kg once-daily for 60 days orally	Allicin: 50 mg\kg, daily for 60 days, orally	Rat	MDA CAT	Allicin could improve sperm parameters (count, motility, and viability) and testicular weight.	[114]
10, 20 mg/kg daily for 5 days IP	Lemon Fruit Extract (LFE): 10 ml/kg, daily for 5 days, orally	Mouse	–	Treatment with LFE could decrease degeneration, atrophy, and spermatogenesis arrest in seminiferous tubules.	[115]
15 mg/kg per week for 8 weeks, orally	Crocin (CR): 10 and 20 mg/kg per day for 8 weeks, orally	Rat	Caspase-3 CAT GPx LH FSH	CR could decrease testicular apoptosis and increase sperm quality and hormonal levels.	[116]
15 mg/kg per week IP	Gallic acid (GA): 12.5 mg/kg per day, IP, concurrently with CP induction	Mouse	MDA	CP could lead to testicular atrophy, sperm DNA fragmentation, elevated MDA levels. Treatment with GA ameliorated the adverse effects of CP.	[117]
15 mg/kg per week for 35 days IP	GA: 12.5, 25, and 50 mg/kg per day for 35 days, IP, concurrently with CP induction	Mouse	MDA SCD	Treatment with GA could reduce sperm DNA fragmentation, testicular atrophy, and MDA levels.	[118]
15 mg/kg per week for 35 days IP	Ethyl Pyruvate (EP): 40 mg/kg per day for 35 days, IP	Mouse	SOD MDA	EP could increase tubal differentiation and spermiogenesis indexes.	[119]
15 mg/kg once a week for 35 days IP	EP: 40 mg/kg, daily for 35 days, IP	Mouse	TAC ALP	EP could increase the number of Leydig cells and the diameter of seminiferous tubules.	[120]
15 mg/kg, once a week for 4 weeks IP	Zinc Oxide Nanoparticles (nZno): 5 mg/kg, daily for 4 weeks, IP	Mouse	–	CP could reduce the numbers of primary spermatocyte, spermatid, and spermatozoa cells, and the thickness of the seminiferous tubules. Treatment with nZno prevented these changes induced by CP.	[121]
15 mg/kg once a week for 6 weeks orally	Fennel Essential Oil (FEO): 1 mg/kg once a week for 6 weeks, orally, 2 h after CP induction	Rat	Casepase-3 LH MDA CAT SOD GSH	CP decreased the diameter and germinal epithelial height of the seminiferous tubules and also led to the degeneration of germ cells and cytoplasmic vacuolation. Treatment with FEO could elevate the concentration of hormones and improve the histological structure of the testis.	[122]
15 mg/kg once a week for 10 weeks IP	SIP: 80 mg/kg, daily for 10 weeks, orally	Mouse	LDH MDA GSH GPX GST SOD CAT NO	SIP could prevent serious negative changes in sexual hormone contents, histopathological features, and sperm parameters.	[123]
15 mg/kg, once a week for 10 weeks IP	Squid ink polysaccharides (SIP): 80 mg/kg once a day for 10 weeks, IP	Mouse	–	SIP could reduce abnormal rates of sperm and fetus and increase total antioxidant capacity in the testes.	[124]
15 mg/kg once a week for 10 weeks IP	SIP: 80 mg/kg once a day for 10 weeks, IP	Mouse	NQO-1 HDAC2	SIP via activating Nrf2 could have preventive roles in CP-treated testicles.	[125]

(*continued on next page*)

Table 5 (*continued*)

Doses/duration, route administration	Antioxidant, doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
20 mg/kg, daily for 5 days IP	Oyster Peptides and Oyster Powder: 0.2 g/kg daily for 5 days, orally	Rat	MDA SOD P450scc SF-1 StAR LH FSH TAC	Treatment with oyster peptides and oyster powder could ameliorate CP-induced reproductive impairment in male rats.	[126]
28 mg/kg daily for 5 days IP	Lycium barbarum: 0.2 g/kg, 0.4 g/kg and 0.6 g/kg, daily for 5 days, orally	Rat	SOD NO	LBP could improve sperm density and movement and also decrease the NO level in the testes against CP.	[127]
30 mg/kg daily for 28 days orally	Ethanolic Seed Extract of Telfaira Occidentalis (ESETO): 100 and 1000 mg/kg, daily for 28 days, orally	Rat	TH FSH	ESETO could increase both body and testicular weights and serum levels of testosterone and FSH in a model of CP-induced reproductive toxicity.	[128]
35 mg/kg daily for 5 days IP	L-Carnitine (LC): 2.1 ml/kg daily for 5 days, orally	Rat	LC3 Beclin-1 Estradiol	Treatment with LC could decrease germ cell apoptosis and increased testosterone level and sperm motility and viability.	[129]
50 mg/kg 1 dose and 2 doses day one 1st dose and day eight 2nd dose IP	–	Rat	–	CP could elevate the testicular injury.	[102]
50 mg/kg single dose IP	Garlic oil (GO): 10% wt/wt for 21 days, IP	Rat	CAT SOD GPx GSH MDA LH FSH	GO could protect the testis from CP toxicity via its antioxidant property and enhance hormone levels (testosterone, FSH, and LH).	[130]
50 mg/kg single dose IP	Moringa oleifera Leaf Extract (MOE): 25 mg/kg single dose, IP, 24 h before CP induction	Mouse	MDA GSH SOD CAT	CP could increase sperm head abnormality and DNA damage. MOE could increase sperm density and motility.	[105]
60 mg/kg once a day for 5 days IP	LC: 100 mg/kg once a day for 5 days, IP, 30 min before CP induction	Mouse	TGF-β3 GDNF	CP decreased some parameters of sperm, induced spermatogenesis arrest, and led to damage or efficacy to testis Sertoli cells. Administration of LC could ameliorate the adverse effects of CP on reproductive function.	[131]
65 mg/kg single dose IP	Aqueous Extract of Amaranthus viridis (AEAVL): 100, 200, and 400 mg/kg, orally; Vit E: 100 mg/kg, daily for 30 days, orally, 5 days after CP induction	Rat	FSH LH MDA GSH	AEAVL could improve some sperm parameters, hormonal levels, and activity of the antioxidant system against CP.	[132]
100 mg/kg single dose IP	Olive Leaf Extract (OLE) and Oleuropein: 100 mg/kg, daily for 28 days, IP	Mouse	LH FSH MDA TAC	CP could increase oxidative stress and the number of abnormal sperm. Treatments with Oleuropein and/or OLE could ameliorate the harmful effects of CP on the male reproductive system.	[133]
100 mg/kg single dose IP	Ginger Extract: 500 mg/kg, daily for 35 days, orally, 35 days before CP induction	Rat	Caspase-3	CP could decrease the diameter of the seminiferous tubules and disrupt the integrity of the testicular architecture. Treatment with ginger could improve the structure of germinal epithelium and cellular attachments.	[134]
100 mg/kg single dose IP	Green tea: 2500 mg/kg, daily for 14 days, orally, 1 h before CP induction	Mouse	17β-HSD GPx GST	Pretreatment with green tea could reduce DNA damage, restored 17β-HSD activity, and increase sperm concentration in CP-induced reproductive toxicity.	[135]
100 mg/kg single dose IP	Tribulus terrestris (TT): 11 mg/kg daily for 14 days, orally, 1 h before CP induction	Mouse	MDA GST 17β-HSD SOD CAT GPx	TT could enhance 17 β-HSD activity, some antioxidant enzymes, and serum testosterone levels in CP-induced reproductive toxicity.	[136]
100 mg/kg one dose in a week for 3 weeks IP	Moringa oleifera Leaves (MOE): 100 mg/kg, 5 doses in a week for 4 weeks, IP, 24 h before CP induction	Mouse	FSH Caspase-3 TAC	CP decreased some sperm parameters and testosterone and inhibin B levels, and increased DNA damage in spermatozoa and FSH level. Administration of MOE by modulating the expression of genes specific to Sertoli and spermatogenic cells could ameliorate CP-induced damage.	[104]
100 mg/kg once a week for 5 weeks IP	Ghrelin: 80 μg/kg, daily for 5 weeks, IP	Mouse	MDA GSH SOD CAT GPx TAC	CP decreased GPx, SOD, and total antioxidant capacity. Treatment with ghrelin could improve the mentioned factors and reduce degenerative changes in the testicular tissue.	[137]
100 mg/kg once a week for 5 weeks IP	Zn(II)-curcumin: 10 and 30 mg/kg, daily for 5 weeks, orally	Mouse	MDA SOD GSH	Zn(II)-curcumin by reducing histological alterations and improving sperm parameters (sperm count, viability, motility) could ameliorate CP-induced reproductive system impairments.	[138]
120 mg/kg single dose IP	Squid ink polysaccharide (SIP): 40, 60, 80 mg/kg, once a day for 2 weeks, orally	Mouse	SOD CAT		[139]

(*continued on next page*)

Table 5 (*continued*)

Doses/duration, route administration	Antioxidant, doses/duration, route of administration	Species	Targets/ pathways	Observations	Ref.
			MDA LC3 Beclin-1	SIP could decrease characteristics of cell autophagy (chromatin pyknosis, vacuoles, and mitochondrial swelling) in CP-treated Leydig cells.	
150 mg/kg single dose IP	Royal jelly (RJ): 300 mg/kg daily for 14 days, orally, 1 day before CP induction	Rat	MDA GPx eNOS Bax	Concomitant administration of RJ and CP could ameliorate biochemical and histological changes.	[140]
150 mg/kg single dose IP	Curcumin: 20 mg/kg, daily for 14 days, orally, 1 day before CP induction.	Rat	Caspase-3 MDA NO H2O2 LDH ALP	Pre and post-treatment with curcumin could affect the elevation of oxidative stress biomarkers, prevent histo-architectural damages of the epididymis and testicles, and increase the sperm quality and quantity against CP-induced reproductive toxicity.	[141]
150 mg/kg daily for 10 days IP	Diallyl Disulfide (DADS): 50 mg/kg, daily for 10 days, orally	Rat	CYP2B1/2 CYP2C11 CYP3A1 LPO GSH TAC MDA CAT GPx	DADS could attenuate the spermatotoxicity of CP and improve reproductive organ weights.	[142]
200 mg/kg single dose IP	Gallic acid (GA): 60 and 120 mg/kg, daily for 14 days, IP	Rat	LH FSH MDA	GA increased LH, FSH, and testosterone levels, and also decreased testicular and epididymal atrophy.	[101]
200 mg/ml a single dose for 4 days orally	Boron (B): 200 mg/kg for 6 days, orally	Rat	TOC MDA Bax Caspase-3 TAC GSH Bcl-2	CP could increase seminiferous tubule damages and decrease antioxidant status. Treatment with B could ameliorate the deleterious effects of CP.	[143]
200 mg/kg single dose IP	Nerolidol (NER): 200 and 400 mg/kg, for 14 days, orally	Mouse	MDA CAT TNF-α NO MPO	Treatment with nerolidol could prevent histo-architectural damages of testicles, decrease oxidative stress and inflammation, and increase sperm count and testosterone level in a model of CP-induced reproductive toxicity.	[144]
200 mg/kg single dose IP	Aquilaria malaccensis (AM): 100, 300, and 500 mg/kg, orally	Rat	–	Administration of AM in CP exposed rats could increase reproductive organs' weight and reduce the number of dead and abnormal sperm.	[145]
200 mg/kg single dose IP	Chrysin (CH): 25 and 50 mg/kg, daily for 7 days, orally	Rat	–	CH had protective effects against CP-induced testicular toxicity.	[146]
200 mg/kg single dose IP	Cerium Oxide Nanoparticles (NC): 100 μg/kg, daily for 3 days, IP	Mouse	MDA GSH	NC could decrease sperm abnormality, ROS, and spermatogenesis arrest while increasing germinal epithelium thickness and diameter of seminiferous tubules in CP-induced reproductive toxicity.	[147]
200 mg/kg single dose IP	Amifostine (AMF): 200 and 400 mg/kg, daily for 7 days, IP, 1 day before CP induction	Rat	FSH LH	AMF could increase tubular epithelial height, the Johnsen scores, and sperm count while decreasing Sertoli cell damages in a model of CP-induced reproductive toxicity.	[148]
200 mg/kg once a week for 5 weeks IP	Aegle marmelos (AEAM): 400, 500, and 600 mg/kg once a week for 5 weeks, orally	Mouse	SOD CAT	CP reduced gonadosomatic index and some sperm parameters and also induced histopathological changes of the testis. Treatment with AEAM could prevent these changes.	[149]

[172]. N-acetylcysteine has protective effects against sperm abnormalities induced by etoposide [173].

Both human and animal studies have provided invaluable results about the impact of chemotherapeutic agents on male fertility. Investigations in animals have enabled investigators to administer these agents in a more regulated manner and to assess their effects not only in whole animals but also in specific cells and tissues. These models have also facilitated the determination of the developmental periods which are more sensitive to these agents as well as the underlying mechanisms of detrimental effects of chemotherapeutic drugs. On the other hand, human studies are less precise in this regard. Since patients usually receive a combination of chemotherapy agents, it is not possible to identify the relative participation of each chemotherapeutic substance in gonad damage. Moreover, results obtained from human studies might be affected by the age of patients or stage of gonadal development and personalized sensitivity to chemotherapy agents [7].

Epigenetic mechanisms are involved in the mediation of the toxic effects of chemotherapeutic agents on germ cells. Substantial changes in the transcriptome of doxorubicin-treated cells indicate the extent of harmful effects of this agent and necessitate the design of multifaceted therapeutic options beyond sole administration of antioxidants to preserve the germ cells from these detrimental effects. Therefore, this field of study needs further explorative investigations to unravel the molecular cascades and possible intervention modalities. Fig. 3 depicts the overall outcomes of negative effects of chemotherapy on creating impaired spermatogenesis which could, in turn, lead to abnormal spermatozoa and yield excess ROS, which could reduce the antioxidant defenses and results in oxidative stress.

Taken together, chemotherapeutic agents have several detrimental effects on spermatogenesis through different mechanisms including induction of DNA damage. Some of these detrimental effects can be resolved following completion of chemotherapy as being evident by sperm recovery in different time points after chemotherapy. The cumulative administrated dose of the chemotherapeutic agents is possibly

Table 6
Summary of human studies that appraised the effects of anti-cancer treatments on male fertility (CYADIC: cyclophosphamide, doxorubicin, and dacarbazine; CYVADIC: vincristine added to CYADIC; SHBG: sex hormone-binding globulin; TESE: testicular sperm extraction).

Chemotherapy drug	Patients	Findings	Ref.
Cyclophosphamide, doxorubicin, dacarbazine, vincristine	58 patients with Ewing and soft tissue sarcomas	CYADIC and CYVADIC regimens in young men increased the risk of permanent sterility especially when the cumulative dose of cyclophosphamide was greater than 7.5 mg/m^2. These regimens induce azoospermia within 90 days.	[150]
Cyclophosphamide, doxorubicin, vincristine, prednisone, bleomycin	71 patients with non-Hodgkin's lymphoma	Cumulative cyclophosphamide dosages greater than 9.5 g/m2 and Pelvic radiation therapy are associated with a high risk of permanent sterility. Sperm counts declined by cyclophosphamide.	[151]
Cyclophosphamide	26 patients	Spermatogenesis was affected by cyclophosphamide. A follow-up after stopping cyclophosphamide therapy (5 months to 4 years) showed a return of spermatogenesis.	[152]
Prednisone (NOVP)	58 patients with Hodgkin's disease	One month after the start of NOVP chemotherapy, sperm counts declined and spermatogenesis was arrested.	[159]
Mechlorethamine, vinblastine, procarbazine, prednisone	–	Leydig cell dysfunction could occur after chemotherapy. Also, there was a lower mean testosterone/SHBG ratio in the patients.	[160]
Cyclophosphamide, ifosfamide, or chlorambucil	892 patients with nonobstructive azoospermia	In 37% of patients who were azoospermic after chemotherapy, spermatozoa could be retrieved from the testes by TESE.	[161]
6-mercaptopurine, methotrexate, L-asparaginase, cyclophosphamide, doxorubicin	25 boys with acute lymphocytic leukemia	During childhood, exposure to alkylating agents led to reducing tubular diameter, interstitial fibrosis, and Sertoli cell damage.	[155]
Alkylating agents	18 patients with malignancy and 14 patients without malignancy	In prepubertal patients exposed to alkylating agents, a long-term reduction of the spermatogonial stem cells (SSC) pool was observed.	[162]
Cyclophosphamide	248 adult male with various cancers	Cyclophosphamide reduced sperm counts, increased FSH levels, which is indirectly reflecting Sertoli cell alteration, and decreased Inhibin B levels. Cyclophosphamide with a dosage of 10 g/m^2 or more could be involved in severe spermatogenic dysfunction.	[163]
Cyclophosphamide, vincristine, prednisone, procarbazine, doxorubicin	75 boys with Hodgkin's disease	Testicular dysfunction, elevation in FSH and LH	[164]
Cisplatin	145 patients with testicular cancer	At the end of chemotherapy, FHS and the number of chemotherapy cycles (4 cycles) were considered as independent factors related to post-chemotherapy normalization of semen findings.	[165]
Cisplatin	178 patients with testicular cancer	After cisplatin treatment, long-term infertility with azoospermia or oligospermia was related to baseline sperm counts. Also, in most patients (80%), their spermatogenesis was recovered by 5 years from treatment.	[166]
Cisplatin, carboplatin	212 consecutive subjects (testicular germ cells tumor)	After 12 and 24 months of cisplatin treatment, an increase in sperm aneuploidy and DNA alterations and a reduction in sperm concentration were observed.	[167]
Doxorubicin	Sperm samples from healthy donors	A reduction in sperm head DNA and an increase in the tail moment (as indicators of DNA damage) were observed.	[153]

the most important determinant of sperm recovery. Although the beneficial effects of antioxidants on attenuation of sperm damage has not been verified in large cohorts of human subjects, based on the results of animal studies and investigations in small cohorts of patients, one can deduce that administration of antioxidants can fasten the pace of sperm recovery following completion of chemotherapy.

It is worth mentioning that the administration of antineoplastic agents results in oxidative stress, this is, the production of free radicals and other ROS. Oxidative stress reduces the rate of cell proliferation, and this fact may interfere with the cytotoxic effects of antineoplastic agents, which depend on the rapid proliferation of cancer cells for optimal activity. Antioxidants detoxify ROS, therefore removing cancer-promoting ROS. Yet, extensive reduction in ROS levels might support tumor proliferation and migration while diminishing some of the negative effects of ROS in neoplastic cells, such as DNA damage [174]. Therefore, identification of the proper dose of antioxidants is so important in benefiting from the preservative effects of these substances on male fertility while do not interfere with cancer chemotherapy.

Another issue that should be addressed in further studies is the effects of anti-cancer therapies on congenital malformations in the offspring of patients who received these agents. A nationwide study of patients with testicular germ cell cancers has reported no additional elevated risk of congenital malformations in children of men who received radiotherapy or chemotherapy. Yet, paternal testicular germ cell tumor by itself has been associated with a modestly higher possibility of malformations in offspring [175]. Additional studies in other

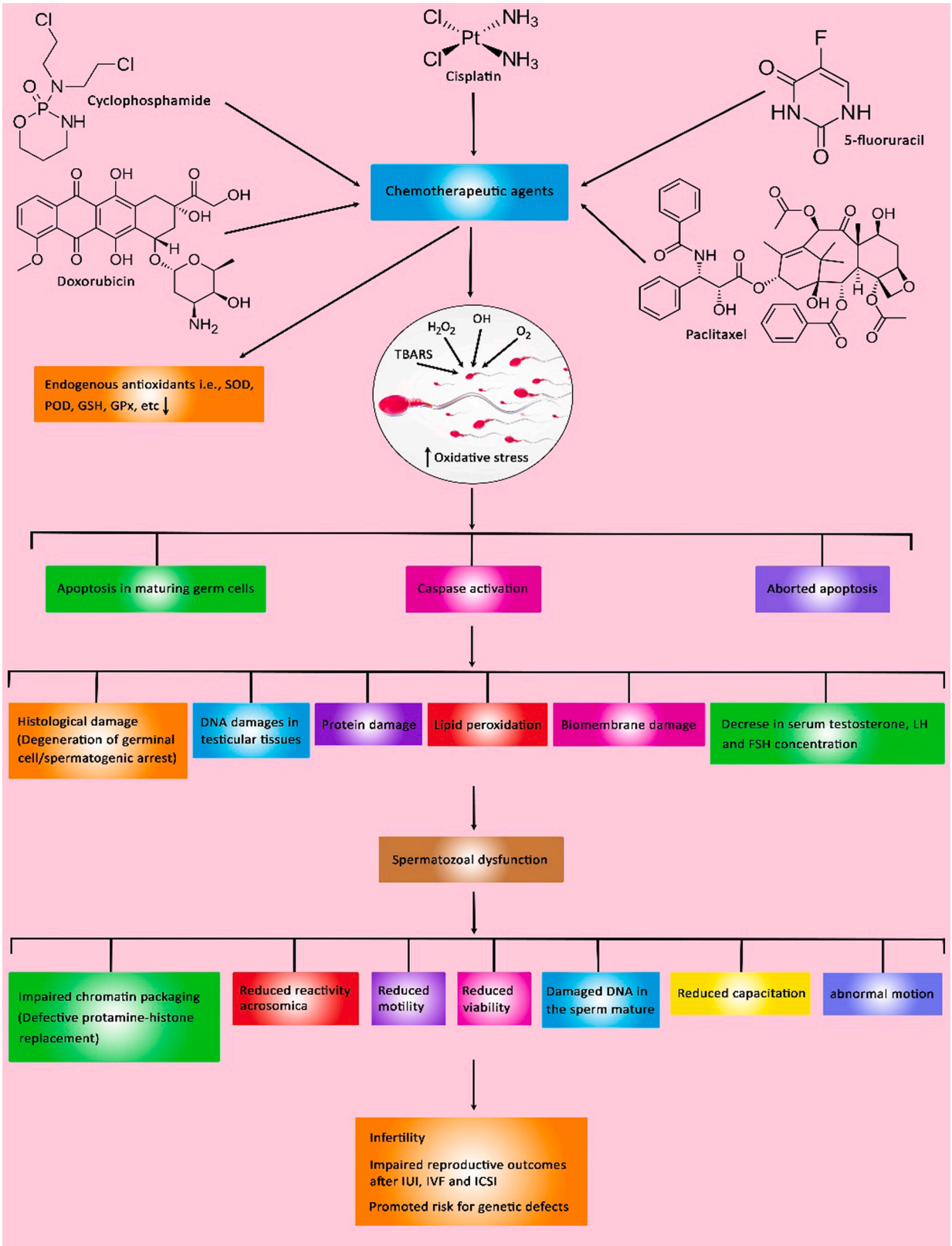

Fig. 3. Schematic explanation of the side effects of chemotherapeutic agents on reproduction and male fertility. Chemotherapeutic agents such as Cisplatin, Doxorubicin, Paclitaxel, 5-Fluorouracil, as well as Cyclophosphamide could have an effective part in reducing the expression of endogenous antioxidants such as SOD, POD, GPx, etc and promoting the level of oxygen species in testes. These factors cause DNA damage to the sperm during spermiation or within its transit via the male reproductive tract and eventually result in oxidative stress-induced sperm dysfunction and male infertility. DNA damage leads to abortive apoptosis, abnormalities, or mutations in sperm maturation and enhances the risk of genetic defects.

types of cancers are needed to appraise whether anti-cancer therapy raises malformations.

Moreover, human studies have shown that alkylating agents and radiotherapy induce mutations in spermatozoa produced in the period of 1–2 weeks following the commencement of treatment. This time has been estimated to be weeks 5–7 for topoisomerase II inhibitors and weeks 7–10 for nucleoside analogs, antimetabolites, and bleomycin [176]. These time points should be considered for sperm retrieval.

Taken together, scarcity of human studies and lack of sufficient data regarding the mechanism of detrimental effects of chemotherapeutic agents in human subjects are limitations of studies in this field.

Author's statement

MT and SGF wrote the draft and revised it. HS, AB, FK, SAA, MS and MM performed the data collection and designed the tables and figures. All the authors read and approved the submitted version.

Conflict of interest statement

The authors declare they have no conflict of interest.

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References

[1] I. Vakalopoulos, P. Dimou, I. Anagnostou, T. Zeginiadou, Impact of cancer and cancer treatment on male fertility, Hormones 14 (4) (2015) 579–589.
[2] W.H.B. Wallace, R.A. Anderson, D.S. Irvine, Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol. 6 (4) (2005) 209–218.
[3] L. Marcon, X. Zhang, B.F. Hales, M.C. Nagano, B. Robaire, Development of a short-term fluorescence-based assay to assess the toxicity of anticancer drugs on rat stem/progenitor spermatogonia in vitro, Biol. Reprod. 83 (2) (2010) 228–237.
[4] M. Liu, B.F. Hales, B. Robaire, Effects of four chemotherapeutic agents, bleomycin, etoposide, cisplatin, and cyclophosphamide, on DNA damage and telomeres in a mouse spermatogonial cell line, Biol. Reprod. 90 (4) (2014) 72, 1-10.
[5] L.C. Lirdi, T. Stumpp, E. Sasso-Cerri, S.M. Miraglia, Amifostine protective effect on cisplatin-treated rat testis, Anat. Rec. 291 (7) (2008) 797–808.
[6] A.P.A. Favareto, C.D.B. Fernandez, D.A.F. da Silva, J.A. Anselmo-Franci, W.D. G. Kempinas, Persistent impairment of testicular histology and sperm motility in adult rats treated with cisplatin at peri-puberty, Basic Clin. Pharmacol. 109 (2) (2011) 85–96.
[7] C.M. Allen, F. Lopes, R.T. Mitchell, N. Spears, How does chemotherapy treatment damage the prepubertal testis? Reproduction 156 (6) (2018) R209–R233.
[8] S. Dasari, P.B. Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action, Eur. J. Pharmacol. 740 (2014) 364–378.
[9] V. Shokri, C. Jalili, F. Raissi, N. Akhshi, A. Ghanbari, Evaluating the effects of acacetin versus a low dose of cisplatin drug on male reproductive system and kidney in mice: with emphasis on inflammation process, Andrologia 52 (1) (2020) 13444.
[10] F. Shaker Kata, Effect of Cisplatin drug on sperm characterizes, spermatogenesis and sex hormones levels of male mice, Basrah J. Vet. Res. Sci. 39 (2A) (2013) 112–124.
[11] A. Brozovic, A. Ambriović-Ristov, M. Osmak, The relationship between cisplatin-induced reactive oxygen species, glutathione, and BCL-2 and resistance to cisplatin, Crit. Rev. Toxicol. 40 (4) (2010) 347–359.
[12] M. Liu, B.F. Hales, B. Robaire, Effects of four chemotherapeutic agents, bleomycin, etoposide, cisplatin, and cyclophosphamide, on DNA damage and telomeres in a mouse spermatogonial cell line, Biol. Reprod. 90 (4) (2014) 72, 1-10.
[13] E. Smart, F. Lopes, S. Rice, B. Nagy, R.A. Anderson, R.T. Mitchell, N. Spears, Chemotherapy drugs cyclophosphamide, cisplatin and doxorubicin induce germ cell loss in an in vitro model of the prepubertal testis, Sci. Rep. 8 (1) (2018) 1–5.
[14] Y.C. Yadav, Effect of cisplatin on pancreas and testies in Wistar rats: biochemical parameters and histology, Heliyon 5 (8) (2019) 02247.
[15] A. Amin, A.A. Hamza, Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats, Asian J. Androl. 8 (5) (2006) 607–612.
[16] K.M. Saad, R.S. Abdelrahman, E. Said, Mechanistic perspective of protective effects of nilotinib against cisplatin-induced testicular injury in rats: role of JNK/caspase-3 signaling inhibition, Environ. Toxicol. Pharmacol. 76 (2020), 103334.
[17] I.H. Hassan, R.E. Mahmoud, M. Amoura, A. Abdelalim, Effects of adriamycin, cisplatin, and 5-fluorouracil on the testes of albino rats, Br. J. Med. Health Sci. 1 (2013) 45–62.
[18] E. Dere, D.J. Spade, S.J. Hall, A. Altemus, J.D. Smith, J.A. Phillips, J.S. Moffit, K. T. Blanchard, K. Boekelheide, Identification of sperm mRNA biomarkers associated with testis injury during preclinical testing of pharmaceutical compounds, Toxicol. Appl. Pharmacol. 320 (2017) 1–7.
[19] S.E. Elballat, Protective effect of curcumin and vitamin C each alone and in combination on cisplatin-induced sperm abnormalities in male albino rats, J. Basic Appl. Zool. 76 (2016) 52–59.
[20] T. Mercantepe, D. Unal, L. Tumkaya, Z.A. Yazici, Protective effects of amifostine, curcumin and caffeic acid phenethyl ester against cisplatin-induced testis tissue damage in rats, Exp. Ther. Med. 15 (4) (2018) 3404–3412.
[21] S. Razavi, F. Khadivi, F. Hashemi, A. Bakhtiari, Effect of zinc on spermatogenesis and sperm chromatin condensation in bleomycin, etoposide, cisplatin treated rats, Cell J. 20 (4) (2019) 521–526.
[22] M. Al-Bader, N. Kilarkaje, Effects of bleomycin, etoposide and cisplatin treatment on Leydig cell structure and transcription of steroidogenic enzymes in rat testis, Eur. J. Pharmacol. 747 (2015) 150–159.
[23] S. Razavi, F. Hashemi, F. Khadivi, A. Bakhtiari, A. Mokhtarian, H. Mirzaei, Improvement of rat sperm chromatin integrity and spermatogenesis with omega 3 following bleomycin, etoposide and cisplatin treatment, Nutr. Cancer 73 (3) (2021) 514–522.
[24] F. Hashemi, S. Razavi, F. Khadivi, The protective effects of omega3 on ubiquitination and protamination of rat sperm after bleomycin, etoposide, and cisplatin treatment, Nutr. Cancer 70 (8) (2018) 1308–1314.
[25] V.E. Oigbochie, T.E. Lawal, C.D. Ekpruke, Effect of human menopausal gonadotropin on rat testes damaged by cisplatin treatment, Afr. Sci. 18 (1) (2017) 33–41.
[26] A. Aminsharifi, P. Hekmati, A. Noorafshan, S. Karbalay-Doost, E. Nadimi, A. Aryafar, O.K. Hosseinabadi, M.M. Naseri, M. ZarePoor, Scrotal cooling to protect against cisplatin-induced spermatogenesis toxicity: preliminary outcome of an experimental controlled trial, Urology 91 (2016) 90–98.
[27] A.A. Shati, Resveratrol improves sperm parameter and testicular apoptosis in cisplatin-treated rats: effects on ERK1/2, JNK, and Akt pathways, Syst. Biol. Reprod. Med. 65 (3) (2019) 236–249.
[28] O. Yaman, Y. Topcu-Tarladacalisir, L-carnitine counteracts prepubertal exposure to cisplatin induced impaired sperm in adult rats by preventing germ cell apoptosis, Biotech. Histochem. 93 (3) (2018) 157–167.
[29] H. Anand, M.M. Misro, S.B. Sharma, S. Prakash, Protective effects of Eugenia jambolana extract versus N-acetyl cysteine against cisplatin-induced damage in rat testis, Andrologia 47 (2) (2015) 194–208.
[30] R. Ksouri, S. Rabah, S. Mezghani, S. Hamlaoui, The protective effect of grape seed and skin extract and Ulva rigida againstoxidative stress induced by cisplatin on the testis of rats, SAUJS 24 (2) (2020) 312–323.
[31] Z. KESHTMAND, S. ORYAN, A. GHANBARI, M. KHAZAEI, Protective effect of Tribulus terrestris hydroalcoholic extract against cisplatin-induced cytotoxicity on sperm parameters in male mice, Int. J. Morphol. 32 (2) (2014) 551–557.
[32] Z. Keshtmand, A. Ghanbari, R. Keshtmand, Protective effect of Tribulus terrestris hydroalcoholic extract against cisplatin – induce apoptisis on testis in mice, Anat. Sci. J. 11 (2) (2014) 75–80.
[33] B.A. Abdel-Wahab, S.A. Alkahtani, E.A.M. Elagab, Tadalafil alleviates cisplatin-induced reproductive toxicity through the activation of the Nrf2/HO-1 pathway and the inhibition of oxidative stress and apoptosis in male rats, Reprod. Toxicol. 96 (2020) 165–174.
[34] V.U. Nna, G.A. Ujah, J.B. Suleiman, M. Mohamed, C. Nwokocha, T.J. Akpan, H. C. Ekuma, V.V. Fubara, C.B. Kekung-Asu, E.E. Osim, Tert-butylhydroquinone preserve testicular steroidogenesis and spermatogenesis in cisplatin-intoxicated rats by targeting oxidative stress, inflammation and apoptosis, Toxicology 441 (2020), 152528.
[35] S. Jahan, A. Munawar, S. Razak, S. Anam, Q.U. Ain, H. Ullah, T. Afsar, M. Abulmeaty, A. Almajwal, Ameliorative effects of rutin against cisplatin-induced reproductive toxicity in male rats, BMC Urol. 18 (1) (2018) 107.
[36] M. Aldemir, E. Okulu, K. Kosemehmetoglu, K. Ener, F. Topal, O. Evirgen, E. Gurleyik, A. Avci, Evaluation of the protective effect of quercetin against cisplatin-induced renal and testis tissue damage and sperm parameters in rats, Andrologia 46 (10) (2014) 1089–1097.
[37] K.P. Reddy, P. Madhu, P.S. Reddy, Protective effects of resveratrol against cisplatin-induced testicular and epididymal toxicity in rats, Food Chem. Toxicol. 91 (2016) 65–72.
[38] Z. Yanmeng, Z. Hailian, G. Liping, Anti-apoptotic effect of grape seed proanthocyanidin extract on cisplatin-induced apoptosis in rat testis, Food Sci. Technol. Res. 21 (6) (2015) 805–811.
[39] S. Saral, E. Ozcelik, A. Cetin, O. Saral, N. Basak, M. Aydin, O. Ciftci, Protective role of Diospyros lotus on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats, Andrologia 48 (3) (2016) 308–317.
[40] K. Kaya, O. Ciftci, M. Aydin, A. Cetin, N. Basak, Favourable effect of β-glucan treatment against cisplatin-induced reproductive system damage in male rats, Andrologia 51 (9) (2019) 13342.
[41] G.H. Heeba, A.A. Hamza, S.O. Hassanin, Induction of heme oxygenase-1 with hemin alleviates cisplatin-induced reproductive toxicity in male rats and enhances its cytotoxicity in prostate cancer cell line, Toxicol. Lett. 264 (2016) 38–50.
[42] E.H. Aksu, O. Akman, M. Özkaraca, A. Ömür, Ö.M. Uçar, Effect of Maclura pomifera extract on cisplatin-induced damages in reproductive system of male rats, Kafkas Univ. Vet. Fak. Derg. 21 (2015) 397–403.

[43] H. Eren, T. Mercantepe, L. Tumkaya, F. Mercantepe, E. Dil, M.O. Horsanali, A. Yilmaz, Evaluation of the protective effects of amifostine and melatonin against cisplatin induced testis injury via oxidative stress and apoptosis in rats, Exp. Mol. Pathol. 112 (2020), 104324.
[44] M. Tian, F. Liu, H. Liu, Q. Zhang, L. Li, X. Hou, J. Zhao, S. Li, X. Chang, Y. Sun, Grape seed procyanidins extract attenuates Cisplatin-induced oxidative stress and testosterone synthase inhibition in rat testes, Syst. Biol. Reprod. Med. 64 (4) (2018) 246–259.
[45] A.A. Fouad, H.O. Qutub, A.E.A. Fouad, A.M. Audeh, W.N. Al-Melhim, Epigallocatechin-3-gallate counters cisplatin toxicity of rat testes, Pharm. Biol. 55 (1) (2017) 1710–1714.
[46] E.H. Aksu, F.M. Kandemir, S. Altun, S. Kucukler, S. Comakli, A.D. Omur, Ameliorative effect of carvacrol on cisplatin-induced reproductive damage in male rats, J. Biochem. Mol. Toxicol. 30 (10) (2016) 513–520.
[47] M. Ijaz, M. Khan, S. Yousaf, S. Nasir, H. Naz, H. Anwar, T. Younis, A. Samad, Methanolic extract of Fraxinus xanthoxyloides attenuates cisplatin-induced reproductive toxicity in male albino rats, Pak. Vet. J. (1–5) (2020).
[48] N. Coskun, M.T. Hatipoglu, C. Ozogul, C. Korkmaz, S.N. Akyol, S.C. Micili, G. S. Arik, D. Erdogan, The protective effects of acetyl L-carnitine on testis gonadotoxicity induced by Cisplatin in rats, Balk. Med. J. 30 (2) (2013) 235–241.
[49] C.F. Thorn, C. Oshiro, S. Marsh, T. Hernandez-Boussard, H. McLeod, T.E. Klein, R. B. Altman, Doxorubicin pathways: pharmacodynamics and adverse effects, Pharmacogenet. Genom. 21 (7) (2011) 440–446.
[50] D.A. Gewirtz, A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin, Biochem. Pharmacol. 57 (7) (1999) 727–741.
[51] O.O. Akinjo, T.W. Gant, E.L. Marczylo, Perturbation of epigenetic processes by doxorubicin in the mouse testis, Toxicol. Res. 5 (4) (2016) 1229–1243.
[52] K. Sakai, M. Ideta-Otsuka, H. Saito, Y. Hiradate, K. Hara, K. Igarashi, K. Tanemura, Effects of doxorubicin on sperm DNA methylation in mouse models of testicular toxicity, Biochem. Biophys. Res. Commun. 498 (3) (2018) 674–679.
[53] Z.A. Ahmed, A.N. Abtar, H.H. Othman, T.A. Aziz, Effects of quercetin, sitagliptin alone or in combination in testicular toxicity induced by doxorubicin in rats, Drug Des. Dev. Ther. 13 (2019) 3321–3329.
[54] A.R. Tremblay, G. Delbes, In vitro study of doxorubicin-induced oxidative stress in spermatogonia and immature Sertoli cells, Toxicol. Appl. Pharmacol. 348 (2018) 32–42.
[55] N. Özyılmaz Yay, G. Şener, F. Ercan, Resveratrol treatment reduces apoptosis and morphological alterations in cisplatin induced testis damage, J. Res. Pharm. 23 (4) (2019) 621–631.
[56] A.A. Haq, S. Nigar, D. Rehman, Potential of ascorbic acid as antioxidant on chemotherapeutic agent induced change in morphology of testes, J. Pak. Med. Assoc. 67 (4) (2017) 586–589.
[57] R.E. Cabral, F.K. Okada, T. Stumpp, V. Vendramini, S.M. Miraglia, Carnitine partially protects the rat testis against the late damage produced by doxorubicin administered during pre-puberty, Andrology 2 (6) (2014) 931–942.
[58] E. Taskin, C. Guven, S.T. Kaya, L. Sahin, S. Kocahan, A.Z. Degirmencioglu, F. M. Gur, Y. Sevgiler, The role of toll-like receptors in the protective effect of melatonin against doxorubicin-induced pancreatic beta cell toxicity, Life Sci. 233 (2019), 116704.
[59] P.P. Trivedi, S. Kushwaha, D.N. Tripathi, G.B. Jena, Evaluation of male germ cell toxicity in rats: correlation between sperm head morphology and sperm comet assay, Mutat. Res. 703 (2) (2010) 115–121.
[60] J. Magalhaes, A. Ascensao, A.I. Padrao, I.M. Aleixo, E. Santos-Alves, S. Rocha-Rodrigues, A. Ferreira, L. Korrodi-Gregorio, R. Vitorino, R. Ferreira, M. Fardilha, Can exercise training counteract doxorubicin-induced oxidative damage of testis proteome? Toxicol. Lett. 280 (2017) 57–69.
[61] R.H. Mohamed, R.A. Karam, H.A. Hagrass, M.G. Amer, M.R. Abd El-Haleem, Anti-apoptotic effect of spermatogonial stem cells on doxorubicin-induced testicular toxicity in rats, Gene 561 (1) (2015) 107–114.
[62] Y.C. Yeh, T.J. Liu, L.C. Wang, H.W. Lee, C.T. Ting, W.L. Lee, C.J. Hung, K. Y. Wang, H.C. Lai, H.C. Lai, A standardized extract of Ginkgo biloba suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes, Br. J. Pharmacol. 156 (1) (2009) 48–61.
[63] J. Das, J. Ghosh, P. Manna, P.C. Sil, Taurine protects rat testes against doxorubicin-induced oxidative stress as well as p53, Fas and caspase 12-mediated apoptosis, Amino Acids 42 (5) (2012) 1839–1855.
[64] A.M. Kabel, Zinc/alogliptin combination attenuates testicular toxicity induced by doxorubicin in rats: role of oxidative stress, apoptosis and TGF-beta1/NF-kappaB signaling, Biomed. Pharmacother. 97 (2018) 439–449.
[65] J. Kang, Y. Lee, K. No, E. Jung, J. Sung, Y. Kim, S. Nam, Ginseng intestinal metabolite-I (GIM-I) reduces doxorubicin toxicity in the mouse testis, Reprod. Toxicol. 16 (3) (2002) 291–298.
[66] S.V. Ramanjaneyulu, P.P. Trivedi, S. Kushwaha, A. Vikram, G.B. Jena, Protective role of atorvastatin against doxorubicin-induced cardiotoxicity and testicular toxicity in mice, J. Physiol. Biochem. 69 (3) (2013) 513–525.
[67] Y.C. Yeh, H.C. Lai, C.T. Ting, W.L. Lee, L.C. Wang, K.Y. Wang, H.C. Lai, T.J. Liu, Protection by doxycycline against doxorubicin-induced oxidative stress and apoptosis in mouse testes, Biochem. Pharmacol. 74 (7) (2007) 969–980.
[68] M. Boussada, R.B. Ali, A.B. Said, K. Bokri, A.B. Akacha, C. Dziri, M.V. El May, Selenium and a newly synthesized Thiocyanoacetamide reduce Doxorubicin gonadotoxicity in male rat, Biomed. Pharmacother. 89 (2017) 1005–1017.
[69] M.H. Abdelaziz, E.Y. Salah El-Din, M.H. El-Dakdoky, T.A. Ahmed, The impact of mesenchymal stem cells on doxorubicin-induced testicular toxicity and progeny outcome of male prepubertal rats, Birth Defects Res. 111 (13) (2019) 906–919.
[70] G.S. Georgy, O.W. Maher, Ellagic acid and rosmarinic acid attenuate doxorubicin-induced testicular injury in rats, J. Biochem. Mol. Toxicol. 31 (9) (2017), e21937.
[71] P. Badkoobeh, K. Parivar, S.M. Kalantar, S.D. Hosseini, A. Salabat, Effect of nano-zinc oxide on doxorubicin- induced oxidative stress and sperm disorders in adult male Wistar rats, Iran. J. Reprod. Med. 11 (5) (2013) 355–364.
[72] Z.K. El-Maddawy, W.S.H. Abd El Naby, Protective effects of zinc oxide nanoparticles against doxorubicin induced testicular toxicity and DNA damage in male rats, Toxicol. Res. 8 (5) (2019) 654–662.
[73] R.C. Silva, D.M.C. Britto, W. de Fatima Pereira, G.E.A. Brito-Melo, C.T. Machado, M.M. Pedreira, Effect of short- and medium-term toxicity of doxorubicin on spermatogenesis in adult Wistar rats, Reprod. Biol. 18 (2) (2018) 169–176.
[74] C. Gurel, G.C. Kuscu, A. Buhur, M. Dagdeviren, F. Oltulu, N.U. Karabay Yavasoglu, A. Yavasoglu, Fluvastatin attenuates doxorubicin-induced testicular toxicity in rats by reducing oxidative stress and regulating the blood-testis barrier via mTOR signaling pathway, Hum. Exp. Toxicol. 38 (12) (2019) 1329–1343.
[75] N. Baek, O.W. Seo, M. Kim, J. Hulme, S.S. An, Monitoring the effects of doxorubicin on 3D-spheroid tumor cells in real-time, Onco Targets Ther. 9 (2016) 7207–7218.
[76] B. Srdjenovic, V. Milic-Torres, N. Grujic, K. Stankov, A. Djordjevic, V. Vasovic, Antioxidant properties of fullerenol C60(OH)24 in rat kidneys, testes, and lungs treated with doxorubicin, Toxicol. Mech. Methods 20 (6) (2010) 298–305.
[77] I. Vapa, V.M. Torres, A. Djordjevic, V. Vasovic, B. Srdjenovic, V.D. Simic, J. K. Popovic, Effect of fullerenol C(60)(OH) (24) on lipid peroxidation of kidneys, testes and lungs in rats treated with doxorubicine, Eur. J. Drug Metab. Pharmacokinet. 37 (4) (2012) 301–307.
[78] K. Renu, A. Valsala Gopalakrishnan, Deciphering the molecular mechanism during doxorubicin-mediated oxidative stress, apoptosis through Nrf2 and PGC-1alpha in a rat testicular milieu, Reprod. Biol. 19 (1) (2019) 22–37.
[79] E. Ozturk, E. Kaymak, A.T. Akin, D. Karabulut, H.M. Unsal, B. Yakan, Thymoquinone is a protective agent that reduces the negative effects of doxorubicin in rat testis, Hum. Exp. Toxicol. 39 (2020) 1364–1373, 960327120924108.
[80] A.A. Fouad, M.M.M. Refaie, M.I. Abdelghany, Naringenin palliates cisplatin and doxorubicin gonadal toxicity in male rats, Toxicol. Mech. Methods 29 (1) (2019) 67–73.
[81] M.J. Olusoji, O.M. Oyeyemi, E.R. Asenuga, T.O. Omobowale, O.L. Ajayi, A. A. Oyagbemi, Protective effect of Gallic acid on doxorubicin-induced testicular and epididymal toxicity, Andrologia 49 (4) (2017), e12635.
[82] S.K. Shafiei-Roudbari, H. Malekinejad, H. Janbaz-Aciabar, M. Razi, Crosstalk between E2F1 and P53 transcription factors in doxorubicin-induced DNA damage: evidence for preventive/protective effects of silymarin, J. Pharm. Pharmacol. 69 (9) (2017) 1116–1124.
[83] E.A. Salem, N.A. Salem, W.J. Hellstrom, Therapeutic effect of ozone and rutin on adriamycin-induced testicular toxicity in an experimental rat model 49 (1) (2017) e12603.
[84] E.H. Aksu, F.M. Kandemir, S. Yildirim, S. Kucukler, M.B. Dortbudak, C. Caglayan, F. Benzer, Palliative effect of curcumin on doxorubicin-induced testicular damage in male rats, J. Biochem. Mol. Toxicol. 33 (10) (2019) 22384.
[85] S.B. Horwitz, Taxol (paclitaxel): mechanisms of action, Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 5 (Suppl 6) (1994) S3–S6.
[86] S. Sariozkan, G. Turk, M. Guvenc, A. Yuce, S. Ozdamar, F. Canturk, A.H. Yay, Effects of cinnamon (C. zeylanicum) bark oil against taxanes-induced damages in sperm quality, testicular and epididymal oxidant/antioxidant balance, testicular apoptosis, and sperm DNA integrity, Nutr. Cancer 68 (3) (2016) 481–494.
[87] E. Chatzidarellis, N. Makrilia, L. Giza, E. Georgiadis, C. Alamara, K.N. Syrigos, Effects of taxane-based chemotherapy on inhibin B and gonadotropins as biomarkers of spermatogenesis, Fertil. Steril. 94 (2) (2010) 558–563.
[88] S. Sariozkan, G. Turk, A. Eken, L.C. Bayram, A. Baldemir, G. Dogan, Gilaburu (Viburnum opulus L.) fruit extract alleviates testis and sperm damages induced by taxane-based chemotherapeutics, Biomed. Pharmacother. 95 (2017) 1284–1294.
[89] A.M. Abd-Elrazek, H.A. El-Dash, N.I. Said, The role of propolis against paclitaxel-induced oligospermia, sperm abnormality, oxidative stress and DNA damage in testes of male rats, Andrologia 52 (1) (2020) 13394.
[90] F. Delkhoshe-Kasmaie, H. Malekinejad, M. Khoramjouy, A. Rezaei-Golmisheh, H. Janbaze-Acyabar, Royal jelly protects from taxol-induced testicular damages via improvement of antioxidant status and up-regulation of E2f1, Syst. Biol. Reprod. Med. 60 (2) (2014) 80–88.
[91] T.G. Borovskaya, V.E. Goldberg, O.A. Rumpel, A.V. Pahomova, A.V. Perova, E. D. Goldberg, The rat spermatogenesis after injection of paclitaxel (antitumor agent), Bull. Exp. Biol. Med. 147 (6) (2009) 715–718.
[92] U. D'souza, K. Narayana, Induction of seminiferous tubular atrophy by single dose of 5-fluorouracil (5-FU) in Wistar rats, Indian J. Physiol. Pharmacol. 45 (1) (2001) 87–94.
[93] W.-W. Mao, L.-Y. Mao, F. Jiang, H.-J. He, Early morphological changes in the mouse testis induced by 5-fluorouracil, Zhonghua nan ke xue Natl. J. Androl. 15 (12) (2009) 1064–1067.
[94] K.S. Rao, 5-Fluorouracil (5-FU) induces the formation of giant cells and sloughing of seminiferous epithelium in the rat testis, Indian J. Physiol. Pharmacol. 44 (3) (2000) 317–322.
[95] S. Takizawa, I. Horii, Endocrinological assessment of toxic effects on the male reproductive system in rats treated with 5-fluorouracil for 2 or 4 weeks, J. Toxicol. Sci. 27 (1) (2002) 49–56.
[96] H.N. Gad El-Hak, T.I.S. Moawad, G.A.-A. Hafez, Effects of 5-fluorouracil on testes histology and sperm morphology assay in mice, Egypt. Acad. J. Biol. Sci. D Histol. Histochem. 9 (1) (2017) 1–9.

[97] U.J. D'souza, Toxic effects of 5-fluorouracil on sperm count in wistar rats, Malays. J. Med. Sci. MJMS 10 (1) (2003) 43–45.
[98] I. El-Sayyad, A. Hassan, Effects of adriamycin, cisplatin, and 5-fluorouracil on the testes of albino rats, Br. J. Med. Health Sci. 1 (2013) 45–62.
[99] H.I. Al-Sadi, Histopathological study of lung, testis, and urinary bladder of mice treated with 5-fluorouracil, RJS 26 (1) (2017) 9–16.
[100] A. Hall, M. Tilby, Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies, Blood Rev. 6 (3) (1992) 163–173.
[101] A. Oyagbemi, T. Omobowale, A. Saba, I. Adedara, E. Olowu, A. Akinrinde, R. Dada, Gallic acid protects against cyclophosphamide-induced toxicity in testis and epididymis of rats, Andrologia 48 (4) (2016) 393–401.
[102] H.A. Al-Salih, N.M. Al-Sharafi, S.S. Al-Qabi, A.A. Al-Darwesh, The pathological features of cyclophosphamide induced multi-organs toxicity in male wister rats, Syst. Rev. Pharm. 11 (6) (2020) 45–49.
[103] N.A. Al-Niwehee, Effect of cyclophosphamide treatment during the embryonic period on fertility of adult male mice, Iraqi J. Sci. 60 (4) (2019) 706–714.
[104] G. Nayak, A. Rao, P. Mullik, S. Mutalik, S.G. Kalthur, S.K. Adiga, G. Kalthur, Ethanolic extract of Moringa oleifera leaves alleviate cyclophosphamide-induced testicular toxicity by improving endocrine function and modulating cell specific gene expression in mouse testis, J. Ethnopharmacol. 259 (2020), 112922.
[105] G. Nayak, A. Vadinkar, S. Nair, S.G. Kalthur, A.S. D'Souza, P. Shetty, S. Mutalik, M. Shetty, G. Kalthur, S. Adiga, Sperm abnormalities induced by pre-pubertal exposure to cyclophosphamide are effectively mitigated by Moringa oleifera leaf extract, Andrologia 48 (2) (2016) 125–136.
[106] S. Shabanian, F. Farahbod, M. Rafieian, F. Ganji, A. Adib, The effects of Vitamin C on sperm quality parameters in laboratory rats following long-term exposure to cyclophosphamide, J. Adv. Pharm. Technol. Res. 8 (2) (2017) 73–79.
[107] F. Sadeghzadeh, A. Sadeghzadeh, S. Changizi-Ashtiyani, S. Bakhshi, F. J. Mashayekhi, M. Mashayekhi, H. Poorcheraghi, A. Zarei, M. Jafari, The effect of hydro-alcoholic extract of Ceratonia Silique L. on spermatogenesis index in rats treated with cyclophosphamide: an experimental study, Int. J. Reprod. Biomed. 18 (4) (2020) 295–306.
[108] N. Moustafa, M. Abdul-Hamid, K.A. El-Nesr, A.M. Abukhadra, Protective effect of alpha lipoic acid and royal jelly against the side effects of cyclophosphamide in testis of male albino rats, Egypt. J. Histol. 43 (2) (2020) 539–553.
[109] M. Afkhami-Ardakani, S. Hasanzadeh, R. Shahrooz, N. Delirezh, H. Malekinejad, Antioxidant Effects of Spirulina platensis (Arthrospira platensis) on Cyclophosphamide-induced Testicular Injury in Rats, Veterinary Research Forum, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, 2018, p. 35.
[110] G.S. El Gharabawy, E.E.-D.E. Abd Allah, I.M. Amr, M. Elmitwalli, Histological and immunohistochemical study of the effect of cyclophosphamide on testis of male adult albino rats and the possible protective role of vitamin E, Egypt. J. Hosp. Med. 77 (6) (2019) 5930–5946.
[111] H.H. Anan, R.A. Zidan, S.A. Abd El-Baset, M.M. Ali, Ameliorative effect of zinc oxide nanoparticles on cyclophosphamide induced testicular injury in adult rat, Tissue Cell 54 (2018) 80–93.
[112] S.A. Shittu, S.-T. Shittu, O.O. Akindele, O.T. Kunle-Alabi, Y. Raji, Protective action of N-acetylcysteine on sperm quality in cyclophosphamide-induced testicular toxicity in male Wistar rats, JBRA Assist. Reprod. 23 (2) (2019) 83–90.
[113] A. Hosseini, S. Zare, Z. Borzouei, F.G. Pakdel, Cyclophosphamide-induced testicular toxicity ameliorate by American ginseng treatment: an experimental study, Int. J. Reprod. Biomed. 16 (11) (2018) 711–718.
[114] S.A.L. Al-Gnami, H.K.A. Al-Mayali, Ameliorating effect of allicin on reproductive functions in cyclophosphamide treated male rats, Plant Arch. 18 (1) (2018) 415–424.
[115] S.M. Quita, Evaluation of lemon fruit extract as an antioxidant agent against histopathological changes induced by cyclophosphamide in the testes of albino mice, Electron. Physician 8 (1) (2016) 1824–1831.
[116] A.G. Potnuri, L. Allakonda, M. Lahkar, Crocin attenuates cyclophosphamide induced testicular toxicity by preserving glutathione redox system, Biomed. Pharmacother. 101 (2018) 174–180.
[117] M.G. Novin, M.G. Golmohammadi, M. Sagha, S.A. Ziai, M.A. Abdollahifar, H. Nazarian, Protective effect of gallic acid on testicular tissue, sperm parameters, and DNA fragmentation against toxicity induced by cyclophosphamide in adult NMRI Mice, Urol. J. 17 (1) (2020) 78–85.
[118] Z. Mehraban, M. Ghaffari Novin, M.G. Golmohammadi, M. Sagha, S.A. Ziai, M. A. Abdollahifar, H. Nazarian, Protective effect of gallic acid on testicular tissue, sperm parameters, and dna fragmentation against toxicity induced by cyclophosphamide in adult NMRI mice, Urol. J. 17 (1) (2020) 78–85.
[119] Z. Bakhtiary, R. Shahrooz, A. Ahmadi, F. Soltanalinejad, Protective Effect of Ethyl Pyruvate on Testicular Histology and Fertilization Potential in Cyclophosphamide Treated Mice, Veterinary Research Forum, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, 2020, pp. 7–13.
[120] Z. Bakhtiary, R. Shahrooz, A. Ahmadi, F. Soltanalinejad, Ethyl pyruvate ameliorates the damage induced by cyclophosphamide on adult mice testes, Int. J. Fertil. Steril. 10 (1) (2016) 79–86.
[121] T. Mohammadi, E. Hoveizi, L. Khajehpour, Z. Jelodar, Protective effects of zinc oxide nanoparticles on testis histological structure in cyclophosphamide treated adult mice, JMUMS 26 (144) (2017) 19–27.
[122] S.A. Sakr, S.Y. Shalaby, R.H. Beder, Protective effect of fennel oil on cyclophosphamide inhibited spermatogenesis andinduced oxidative stress in albino rats, J. Biotechnol. Biomed. Sci. 1 (1) (2017) 1–8.
[123] X. Le, P. Luo, Y. Gu, Y. Tao, H. Liu, Interventional effects of squid ink polysaccharides on cyclophosphamide-associated testicular damage in mice, Bratisl. Lek. Listy 116 (5) (2015) 334–339.
[124] P. Luo, H.-Z. Liu, X.-Y. Le, H. Du, X.-H. Kang, Squid ink polysaccharide prevents chemotherapy induced injury in the testes of reproducing mice, Pak. J. Pharm. Sci. 31 (1) (2018) 25–29.
[125] X. Le, P. Luo, Y. Gu, Y. Tao, H. Liu, Squid ink polysaccharide reduces cyclophosphamide-induced testicular damage via Nrf2/ARE activation pathway in mice, Iran. J. Basic Med. Sci. 18 (8) (2015) 827–831.
[126] L. Jia, S. Huang, X. Yin, Y. Zan, Y. Guo, L. Han, Quercetin suppresses the mobility of breast cancer by suppressing glycolysis through Akt-mTOR pathway mediated autophagy induction, Life Sci. 208 (2018) 123–130.
[127] L. Qian, S. Yu, Protective effect of polysaccharides from Lycium barbarum on spermatogenesis of mice with impaired reproduction system induced by cyclophosphamide, Am. J. Reprod. Immunol. 76 (5) (2016) 383–385.
[128] U.C. Ofoego, E.O. Nweke, C. Orji, Co-administration of cyclophosphamide and ethanolic seed extract of Telfairia occidentalis (Pumpkin) protects testicular functions in adult male albino wistar rats, World J. Pharm. Res. 8 (3) (2019) 325–342.
[129] B. Zhu, Y.-f Zheng, Y.-y Zhang, Y.-s Cao, L. Zhang, X.-g Li, T. Liu, Z.-z Jiao, Q. Wang, Z.-g Zhao, Protective effect of L-carnitine in cyclophosphamide-induced germ cell apoptosis, J. Zhejiang Univ. Sci. B 16 (9) (2015) 780–787.
[130] C.A. Ekeleme-Egedigwe, A.C. Famurewa, E.E. David, C.O. Eleazu, U.O. Egedigwe, Antioxidant potential of garlic oil supplementation prevents cyclophosphamide-induced oxidative testicular damage and endocrine depletion in rats, J. Nutr. Intermed. Metab. 18 (2019), 100109.
[131] Y. Cao, X. Wang, S. Li, H. Wang, L. Yu, P. Wang, The effects of l-carnitine against cyclophosphamide-induced injuries in mouse testis, Basic Clin. Pharmacol. Toxicol. 120 (2) (2017) 152–158.
[132] O.A. Ayoka, O.E. Ojo, E.C. Imafidon, K.A. Ademoye, A.A. Oladele, Neuro-endocrine effects of aqueous extract of Amaranthus viridis (Linn.) leaf in male Wistar rat model of cyclophosphamide-induced reproductive toxicity, Toxicol. Rep. 3 (2016) 608–619.
[133] A. Rostamzadeh, H. Amini-khoei, M.J.M. Korani, M. Rahimi-madiseh, Comparison effects of olive leaf extract and oleuropein compounds on male reproductive function in cyclophosphamide exposed mice, Heliyon 6 (4) (2020) 03785.
[134] H. Ali, A. Ali, S. Al-Ghamdi, G.G. Alanazi, M.A. Alsomait, A.N. Alaskar, A.K. El-Enazi, H.M. Alashqar, G. Ahmad, K. Moawad, Protective effects of ginger extract against the toxicity of cyclophosphamide on testes: an experimental laboratory-based study, Int. J. Med. Health Res. 9 (1) (2020) 27–33.
[135] M.M. Zanchi, V. Manfredini, D. dos Santos Brum, L.M. Vargas, C.C. Spiazzi, M. B. Soares, A.P. Izaguirry, F.W. Santos, Green tea infusion improves cyclophosphamide-induced damage on male mice reproductive system, Toxicol. Rep. 2 (2015) 252–260.
[136] N.F. Pavin, A.P. Izaguirry, M.B. Soares, C.C. Spiazzi, A.S. Mendez, F.G. Leivas, D. dos Santos Brum, F.W. Cibin, Tribulus terrestris protects against male reproductive damage induced by cyclophosphamide in mice, Oxid. Med. Cell. Longev. 2018 (2018) 1–9.
[137] R. Salimnejad, J.S. Rad, D.M. Nejad, Protective effect of ghrelin on oxidative stress and tissue damages of mice testes followed by chemotherapy with cyclophosphamide, Crescent J. Med. Biol. Sci. 5 (2018) 138–143.
[138] W.-P. Lu, X.-T. Mei, Y. Wang, Y.-P. Zheng, Y.-F. Xue, D.-H. Xu, Zn (II)–curcumin protects against oxidative stress, deleterious changes in sperm parameters and histological alterations in a male mouse model of cyclophosphamide-induced reproductive damage, Environ. Toxicol. Pharmacol. 39 (2) (2015) 515–524.
[139] Y.-P. Gu, X.-M. Yang, Z.-H. Duan, J.-H. Shang, P. Luo, W. Xiao, D.-Y. Zhang, H.-Z. Liu, Squid ink polysaccharide prevents autophagy and oxidative stress affected by cyclophosphamide in Leydig cells of mice: a pilot study, Iran. J. Basic Med. Sci. 20 (11) (2017) 1194–1199.
[140] S.M.N. Abdel-Hafez, R.A. Rifaai, W.Y. Abdelzaher, Possible protective effect of royal jelly against cyclophosphamide induced prostatic damage in male albino rats; a biochemical, histological and immuno-histo-chemical study, Biomed. Pharmacother. 90 (2017) 15–23.
[141] S.F. Akomolafe, B.T. Aluko, Protective effect of curcumin on fertility in cyclophosphamide exposed rats: involvement of multiple pathways, J. Food Biochem. 44 (1) (2020) 13095.
[142] S.-H. Kim, I.-C. Lee, J.-W. Ko, I.-S. Shin, C. Moon, S.-H. Kim, J.-D. Heo, J.-C. Kim, Mechanism of protection by diallyl disulfide against cyclophosphamide-induced spermatotoxicity and oxidative stress in rats, Mol. Cell. Toxicol. 12 (3) (2016) 301–312.
[143] M. Cengiz, V. Sahinturk, S.C. Yildiz, İ.K. Şahin, N. Bilici, S.O. Yaman, Y. Altuner, S. Appak-Baskoy, A. Ayhanci, Cyclophosphamide induced oxidative stress, lipid per oxidation, apoptosis and histopathological changes in rats: protective role of boron, J. Trace Elem. Med. Biol. 62 (2020), 126574.
[144] A. Iqubal, M.A. Syed, A.K. Najmi, J. Ali, S.E. Haque, Ameliorative effect of nerolidol on cyclophosphamide-induced gonadal toxicity in Swiss Albino mice: biochemical-, histological-and immunohistochemical-based evidences, Andrologia 52 (4) (2020) 13535.
[145] R.N.H.A. Razak, F. Ismail, M.L.M. Isa, A.Y.A. Wahab, H. Muhammad, R. Ramli, R. A.S.R. Ismail, Ameliorative effects of Aquilaria malaccensis leaves aqueous extract on reproductive toxicity induced by cyclophosphamide in male rats, MJMS 26 (1) (2019) 44–57.
[146] P. Taslimi, F.M. Kandemir, Y. Demir, M. İleritürk, Y. Temel, C. Caglayan, İ. Gulçin, The antidiabetic and anticholinergic effects of chrysin on cyclophosphamide-induced multiple organ toxicity in rats: pharmacological evaluation of some metabolic enzyme activities, J. Biochem. Mol. Toxicol. 33 (6) (2019) 22313.

[147] M. Hamzeh, S.J. Hosseinimehr, A. Karimpour, H.R. Mohammadi, A.R. Khalatbary, F.T. Amiri, Cerium oxide nanoparticles protect cyclophosphamide-induced testicular toxicity in mice, Int. J. Prev. Med. 10 (2019) 1–30.
[148] C. Kaya, A.B. Baseskioglu, S. Yigitaslan, F.Y. Ozatik, O. Ozatik, S. Uslu, The therapeutic potential of amifostine on cyclophosphamide-induced testicular dysfunction in rats: an experimental study, Int. J. Reprod. Biomed. 17 (4) (2019) 245–252.
[149] S. Singh, A.K. Mishra, S. Lata, K.N. Tiwari, Aggravation of cyclophosphamide-induced reproductive toxicity in mice by aqueous extract of Aegle marmelos (L.), Braz. J. Pharm. Sci. 53 (3) (2017) 1–8.
[150] M. Meistrich, G. Wilson, B. Brown, M. Da Cunha, L. Lipshultz, Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas, Cancer 70 (11) (1992) 2703–2712.
[151] R.M. Pryzant, M.L. Meistrich, G. Wilson, B. Brown, P. McLaughlin, Long-term reduction in sperm count after chemotherapy with and without radiation therapy for non-Hodgkin's lymphomas, Am. J. Clin. Oncol. 11 (2) (1993) 239–247.
[152] J. Buchanan, K. Fairley, J. Barrie, Return of spermatogenesis after stopping cyclophosphamide therapy, Lancet 306 (7926) (1975) 156–157.
[153] A. Baumgartner, T. Schmid, E. Cemeli, D. Anderson, Parallel evaluation of doxorubicin-induced genetic damage in human lymphocytes and sperm using the comet assay and spectral karyotyping, Mutagenesis 19 (4) (2004) 313–318.
[154] L. Hyman, E. Gilbert, Testicular atrophy in a prepubescent male after cyclophosphamide therapy, Lancet 300 (7774) (1972) 426–427.
[155] T.W. Hensle, K.A. Burbige, B.R. Shepard, C.C. Marboe, W.A. Blanc, J.H. Wigger, Chemotherapy and its effect on testicular morphology in children, J. Urol. 131 (6) (1984) 1142–1144.
[156] C. Uderzo, A. Locasciulli, R. Marzorati, L. Adamoli, B. Di Natale, G. Nizzoli, M. Cazzaniga, G. Masera, Correlation of gonadal function with histology of testicular biopsies at treatment discontinuation in childhood acute leukemia, Med. Pediatr. Oncol. 12 (2) (1984) 97–100.
[157] J. Penso, B. Lippe, R. Ehrlich, F.G. Smith Jr., Testicular function in prepubertal and pubertal male patients treated with cyclophosphamide for nephrotic syndrome, J. Pediatr. 84 (6) (1974) 831–836.
[158] F. Aubier, F. Flamant, R. Brauner, J.M. Caillaud, J.M. Chaussain, J. Lemerle, Male gonadal function after chemotherapy for solid tumors in childhood, Am. J. Clin. Oncol. 7 (3) (1989) 304–309.
[159] M.L. Meistrich, G. Wilson, K. Mathur, L.M. Fuller, M.A. Rodriguez, P. McLaughlin, J.E. Romaguera, F.F. Cabanillas, C.S. Ha, L.I. Lipshultz, Rapid recovery of spermatogenesis after mitoxantrone, vincristine, vinblastine, and prednisone chemotherapy for Hodgkin's disease, Am. J. Clin. Oncol. 15 (12) (1997) 3488–3495.
[160] S.J. Howell, J.A. Radford, W.D.J. Ryder, S.M. Shalet, Testicular function after cytotoxic chemotherapy: evidence of Leydig cell insufficiency, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 17 (5) (1999) 1493–1498.
[161] W. Hsiao, P.J. Stahl, E.C. Osterberg, E. Nejat, G.D. Palermo, Z. Rosenwaks, P. N. Schlegel, Successful treatment of postchemotherapy azoospermia with microsurgical testicular sperm extraction: the Weill Cornell experience, Am. J. Clin. Oncol. 29 (12) (2011) 1607–1611.
[162] J.-B. Stukenborg, J. Alves-Lopes, M. Kurek, H. Albalushi, A. Reda, V. Keros, V. Töhönen, R. Bjarnason, P. Romerius, M. Sundin, Spermatogonial quantity in human prepubertal testicular tissue collected for fertility preservation prior to potentially sterilizing therapy, Hum. Reprod. 33 (9) (2018) 1677–1683.
[163] N.J. van Casteren, G.H. van der Linden, F.G. Hakvoort-Cammel, K. Hählen, G. R. Dohle, M.M. van den Heuvel-Eibrink, Effect of childhood cancer treatment on fertility markers in adult male long-term survivors, Pediatr. Blood Cancer 52 (1) (2009) 108–112.
[164] J.H. Brämswig, U. Heimes, E. Heiermann, W. Schlegel, E. Nieschlag, G. Schellong, The effects of different cumulative doses of chemotherapy on testicular function. Results in 75 patients treated for Hodgkin's disease during childhood or adolescence, Cancer 65 (6) (1990) 1298–1302.
[165] T. Namekawa, T. Imamoto, M. Kato, T. Sazuka, M. Fuse, S. Sakamoto, K. Kawamura, T. Ichikawa, Testicular function among testicular cancer survivors treated with cisplatin-based chemotherapy, Reprod. Med. Biol. 15 (3) (2016) 175–181.
[166] H. Lampe, A. Horwich, A. Norman, J. Nicholls, D. Dearnaley, Fertility after chemotherapy for testicular germ cell cancers, Am. J. Clin. Oncol. 15 (1) (1997) 239–245.
[167] M. Ghezzi, M. Berretta, A. Bottacin, P. Palego, B. Sartini, I. Cosci, L. Finos, R. Selice, C. Foresta, A. Garolla, Impact of Bep or carboplatin chemotherapy on testicular function and sperm nucleus of subjects with testicular germ cell tumor, Front. Pharmacol. 7 (2016) 122.
[168] P.C. Karmakar, Y.J. Cho, Y.H. Kim, S.E. Jung, J.H. Jin, B.J. Kim, W.S. Kwon, Y. H. Kim, M.G. Pang, B.Y. Ryu, Chemotherapeutic drugs alter functional properties and proteome of mouse testicular germ cells in vitro, Toxicol. Sci. 164 (2) (2018) 465–476.
[169] A. Rabaça, C. Ferreira, R. Bernardino, M. Alves, P. Oliveira, P. Viana, A. Barros, M. Sousa, R. Sá, Use of antioxidant could ameliorate the negative impact of etoposide on human sperm DNA during chemotherapy, Reprod. Biomed. Online 40 (6) (2020) 856–866.
[170] F. Khadivi, S. Razavi, F. Hashemi, Protective effects of zinc on rat sperm chromatin integrity involvement: DNA methylation, DNA fragmentation, ubiquitination and protamination after bleomycin etoposide and cis-platin treatment, Theriogenology 142 (2020) 177–183.
[171] N. Kilarkaje, A.M. Mousa, M.M. Al-Bader, K.M. Khan, Antioxidants enhance the recovery of three cycles of bleomycin, etoposide, and cisplatin-induced testicular dysfunction, pituitary-testicular axis, and fertility in rats, Fertil. Steril. 100 (4) (2013) 1151–1159.
[172] M. Moradi, N. Goodarzi, A. Faramarzi, H. Cheraghi, A.H. Hashemian, C. Jalili, Melatonin protects rats testes against bleomycin, etoposide, and cisplatin-induced toxicity via mitigating nitro-oxidative stress and apoptosis, Biomed. Pharmacother. 138 (2021), 111481.
[173] J. Baetas, A. Rabaça, A. Gonçalves, A. Barros, M. Sousa, R. Sá, Protective role of N-acetylcysteine (NAC) on human sperm exposed to etoposide, Basic Clin. Androl. 29 (1) (2019) 1–9.
[174] H. Yang, R.M. Villani, H. Wang, M.J. Simpson, M.S. Roberts, M. Tang, X. Liang, The role of cellular reactive oxygen species in cancer chemotherapy, J. Exp. Clin. Cancer Res. 37 (1) (2018) 266–274.
[175] Y. Al-Jebari, I. Glimelius, C. Berglund Nord, G. Cohn-Cedermark, O. Ståhl, T. Tandstad, A. Jensen, H. Sagstuen Haugnes, G. Daugaard, L. Rylander, A. Giwercman, Cancer therapy and risk of congenital malformations in children fathered by men treated for testicular germ-cell cancer: a nationwide register study, PLoS Med. 16 (6) (2019), 1002816.
[176] M.L. Meistrich, Risks of genetic damage in offspring conceived using spermatozoa produced during chemotherapy or radiotherapy, Andrology 8 (3) (2020) 545–558.