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Review

The role of melatonin on chemotherapy-induced reproductive toxicity

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Abstract

Objectives Reproductive malfunctions after chemotherapy still are a reason of reducing fertility and need specialized intensive care. The aim of this review was to investigate the effect of melatonin on the reproductive system under threatening with chemotherapeutic drugs.

Methods To find the role of melatonin in the reproductive system during chemotherapy, a full systematic literature search was carried out based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines in the electronic databases up to 17 April 2017 using search terms in the titles and abstracts. A total of 380 articles are screened according to our inclusion and exclusion criteria. Finally, 18 articles were included in this study.

Key findings It has been cleared that melatonin has bilateral effects on reproductive cells. Melatonin protects normal cells via mechanisms, including decrease in oxidative stress, apoptosis, inflammation and modulating mitochondrial function, and sexual hormones. Furthermore, melatonin with antiproliferative properties and direct effects on its receptors improves reproductive injury and function during chemotherapy. On the other hand, melatonin sensitizes the effects of chemotherapeutic drugs and enhances chemotherapy-induced toxicity in cancerous cells through increasing apoptosis, oxidative stress and mitochondrial malfunction.

Conclusions The study provides evidence of the bilateral role of melatonin in the reproductive system during chemotherapy.

Introduction

Nowadays, cancer is one of the major prominent, which affects every society. It causes many deaths worldwide. From 12.7 million cases that cancer encompasses, 7.6 million of them will probably face death in a year.[1] Many investigations have focused on battling cancer with the lowest adverse effects and have made it the most important priority for researchers.[2] Although anticancer drugs are more efficient, they all have adverse effects due to deleterious cytotoxicity against normal cells and tissues in chemotherapy process.[3] The reproductive system is one of the vulnerable organs to chemotherapy. The process of chemotherapy may lead to alteration in fertility, changing in organ structure, sexual hormones, and function and quality of life.[4] Some techniques such as embryo cryopreservation, mature oocyte cryopreservation and ovarian tissue cryopreservation can be applied prior to chemotherapy process.[5] Moreover, these techniques have limitations. They cannot be done in all centres and also, they are invasive to patients.[6] In this regard, many researches are ongoing to discover the effects of some protective agents which can be used in combination with anticancer drugs to mitigate deleterious side effects.[7–9] In this content, using gonadoprotective agents may be useful as an option to increase effectiveness of fertility preservation and reproductive health in patients receiving chemotherapy.[3] Based on the evidence, melatonin in combination with

anticancer drugs has profits and reduces deleterious damages of chemotherapy in the reproductive system.[10,11] Utilization of melatonin has been noticeably increased in various diseases and different organs, indicating the pleiotropy of this molecule.[12] Melatonin, N-acetyl-5-methoxytryptamine, is a lipophilic indoleamine which regulates a variety of physiological functions in the body and numerous reports have evidenced the protective effects of it through multiple pathways on pathological situations.[13–15] Melatonin and its metabolites including cyclic 3-Hydroxymelatonin (3-OHM), N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) were found as vigorous free radical scavengers.[16] Melatonin directly exerts its antioxidant effects through radical adduct formation, single electron transfer and hydrogen transfer.[16,17] Moreover, melatonin indirectly mitigates oxidative stress via upregulation of antioxidant enzymes, downregulation of pro-oxidant enzymes, as well as maintenance of mitochondrial homoeostasis, as the main source of reactive oxidative species (ROS) production.[18,19] 3-OHM as an immediate by-product has more antioxidant power comparing to its ancestor and other metabolites.[20] Melatonin with non-enzymatic way is converted to AFMK and is mainly produced in mitochondria because of its high accumulation.[21] AMK via inhibiting nitric oxide synthase (NOS) activity reduces nitric oxide (NO) both in mitochondria and cytosol.[22] Previous studies demonstrated that melatonin exerts its anti-apoptotic effects through inhibition of mitochondrial permeability transition pore. This exertion results in reduction of releasing factors including cytochrome C, Smac/DIABLO, and the serine protease HtrA2/Omi, apoptosis-inducing factor, endonuclease G and caspase-activated DNAse, which play pivotal roles in triggering apoptosis.[23,24] NO induces release of pro-apoptotic calcium, from mitochondria to cytoplasm. Melatonin, through reducing expression of inducible nitric oxide synthase (iNOS), reduces NO levels.[25–27] Many reports have also shown that melatonin regulates Sertoli cells metabolism during gametes development. Melatonin, due to its amphiphilic properties, acts peripherally through binding to intracellular proteins, nuclear receptors and membrane melatonin receptors including MT1 and MT2.[28] In this regard, melatonin and melatonin receptors act via affecting the secretion of both gonadotropin-releasing hormone and luteinizing hormone (LH) gonadotropins, and testosterone synthesis as well as testicular maturation. Thus, it can improve reproductive function quality.[29] Also, mammalian gametes and embryos are highly vulnerable to oxidative stress due to the presence of high lipid levels. In this case, melatonin prevents reproductive damages through its potent antioxidant and anti-apoptotic ability.[30]

To the best of our knowledge, this is the first systematic review on the protective/sensitizing role of melatonin as a combination therapy in chemotherapy-induced reproductive toxicity. In this content, these questions will be risen: How chemotherapy process destroys the reproductive system? What is the role of melatonin on reproductive toxicity induced by chemotherapy? And what are the mechanisms of this role? In order to make a comprehensive vision of melatonin effects on reproductive cells during chemotherapy process and to answer to the above questions, we aimed to discuss the harmful effects of anticancer drugs on the reproductive system and the mechanisms through which melatonin exerts its role. So, we criticized all studies used melatonin as a combination on reproductive cells following by chemotherapy.

Methods

Search strategy

This systematic review was designed according to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[31] Literature search was performed to identify all available studies in the electronic databases, including PubMed, Web of Science, Scopus and EMBASE up to 17 April 2017, using the following search terms in the titles and abstracts: (melatonin) and (testes or testicular or reproductive or sperm or ovarian or follicle) and (doxorubicin or daunorubicin or idarubicin or cisplatin or carboplatin or oxaliplatin or bleomycin or carmustine or cyclophosphamide or melphalan or etoposide or mitomycin or vinblastine or vinorelbine or paclitaxel or docetaxel or procarbazine or epirubicin or methotrexate or mustine or cytarabine or 5-fluorouracil or capecitabine).

Study selection

Original articles were included that met the following inclusion criteria: (1) studies that used a combination of melatonin and others search term; (2) studies with full-text articles; (3) studies with sufficient information; and (4) no limitation in publications with in-vivo or in-vitro subjects. Exclusion criteria included as follows: (1) case report, (2) letter to the editor, (3) not related abstracts, (4) incomplete data on effects of melatonin on the reproductive system and (5) review articles.

Data extraction

Each eligible article was reviewed and the following information including: (1) authors' names; (2) type of

reproductive injury; (3) models; (4) chemical and dosage as well as route of administration; (5) melatonin dosage and route of administration; (6) chemical outcomes; and (7) melatonin co-administration outcomes were extracted. The extracted data and characteristics of the 18 included studies were summarized in Table 1.

Results

Literature search

Our initial search on multiple databases up to 17 April 2017 resulted in a total of 380 published. Of these, 337 articles were excluded after the title and abstract screening because they did not meet our inclusion criteria. Thus, 43 full-text articles were eligible for full-text assessed and reviewed. Twenty-five articles were eliminated because for non-original article ($n = 3$), incompatible data ($n = 6$), not related studies ($n = 16$). Eventually, 18 remaining articles were included in the systematic review. The study selection process is described in Figure 1.

Protective effects of melatonin from toxicity of chemotherapeutic drugs

Cyclophosphamide (Cytoxan) is a chemotherapeutic alkylating drug, widely used as a medication for its antitumour and immunosuppressant properties.[32] Cyclophosphamide is bioactivated through hepatic microsomal cytochrome P450, and these metabolites are responsible for therapeutic activity. It has been also shown that cyclophosphamide has a wide range of side effects such as testicular toxicity.[33–35] In a study, cyclophosphamide increased lipid peroxidation (LPO) and malondialdehyde (MDA) levels, and reduced catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity as well as glutathione (GSH) level in the testis. Co-administration with melatonin reduced the levels of MDA, thiobarbituric acid reactive substances, and also increased SOD and CAT activity as well as GSH levels.[36,37] Moreover, body and testicular weights were significantly reduced by cyclophosphamide.[37,38] Co-administration of melatonin preserved testicular weight.[37] In histopathological studies, cyclophosphamide induced maturation arrest and depletion in sperms ancestors, congested blood vessels and decreased seminiferous epithelial layers, as well as irregular and diminished tubules containing a few germ cells.[36,37,39] Cyclophosphamide significantly decreased the sperm count and percentage of motile sperms, whereas a significant increase was observed in the percentage of abnormal sperms in rats treated with cyclophosphamide.[37] Co-treatment of melatonin and cyclophosphamide showed a significant protection in spermatogenesis.[37] In addition, cyclophosphamide induced a mild perivascular fibrosis and hyalinization of intertubular tissues which were not observed in the groups received melatonin and cyclophosphamide together.[37,39]

Cisplatin (cis-diamminedichloroplatinum-II) is a highly effective chemotherapeutic drug which is used in various cancers.[40] Cisplatin causes significant side effects on peripheral nerves, head and neck, kidneys, lung, ovarian, testes and myelosuppression.[41,42] Cisplatin treatment significantly increased MDA and GSH levels, it also decreased SOD, CAT and GSH-Px activity in testes.[37,43] Melatonin co-administration decreased MDA, SOD and CAT levels but increased GSH-Px activity and GSH levels.[44] Cisplatin significantly reduced body weight, weight, and size of testis, epididymis, seminal vesicle, prostate, and ovary.[43,45] Co-administration with melatonin normalized body weight, weights, and sizes of testes, epididymis, and prostate.[45] In addition, cisplatin decreased sperm concentration and motility, while abnormal sperms were increased. Co-treatment with melatonin with cisplatin provided moderate normalization of sperm concentration.[44] Cisplatin also induced severe degeneration, necrosis, and interstitial oedema and decreased the diameter of the seminiferous tubule and germinal cell thickness.[43] These changes were partially reversed by treatment with melatonin.[43] Melatonin co-administration with cisplatin and vinblastine, as well as bleomycin, showed partial restoration in different spermatogenic stages with intact basement membrane and marginal increase.[44] Furthermore, cisplatin induced a significant decrease in germ cells, seminiferous epithelial layers and spermatogonia, as well as irregular seminiferous tubules, significant maturation arrest and low Johnsen score in testis.[37] Perivascular fibrosis and hyalinization of intertubular tissues were observed in the rats receiving cisplatin, while melatonin recovered these damages.[37] Moreover, cisplatin alone or in combination with vinblastine and bleomycin decreased plasma testosterone levels and increased follicle stimulating hormone (FSH) and LH levels which is significantly normalized by melatonin.[37,44] Furthermore, cisplatin decreased 3β- and 17β-hydroxysteroid dehydrogenases (HSDs) activity, as marker enzymes for steroidogenesis, in combination with vinblastine and bleomycin. Co-administration of melatonin resulted in significant increase in the HSDs activity.[44] Also, cisplatin elevated abnormal multi folliculogenesis and activated primary follicles, ovarian abnormalities, atrophy, corpus luteum and reduced the number of dormant primordial follicles in the ovary. Co-administration of melatonin significantly improved ovarian abnormalities, the number of primordial follicles and it also suppressed primary follicle formation loss through repressing the activation of primordial follicles into primary follicles.[45] Cisplatin induced apoptosis around the

Table 1 The details of included studies

Author' names	Type of reproductive injury	Models	Chemical & dosage & route of administration	Melatonin dosage & route of administration	Chemical outcomes	Melatonin co-treatment outcomes
Chabra *et al.*[36]	Testes	*In vivo*/Mice	Cycliophospamide & 200 mg/kg & ip	2.5, 5, 10 & 20 mg/kg & ip	↑TBARS & ↓GSH levels, ↓SOD & CAT activity, ↓Different spermatogenic cells	↓TBARS & ↑ GSH levels, ↑SOD & CAT activity, ↑Different spermatogenic cells
Abd-El hameed Edrees *et al.*[39]	Testes	*In vivo*/Rat	Cyclophosphamide & 20, 100 mg/kg & ip	10 mg/kg & ip	↓Body & testicular weight, ↑Spermatogenic separation cells in seminiferous tubules, ↑Congested blood vessels, ↑Cytoplasmic vacuolation in Sertoli cells, ↑Perivascular & peritubular collagen fibres, ↑Distortion in seminiferous tubules, ↑Cytoplasmic vacuolation in spermatogenic cells	↓Distortion of seminiferous tubules, ↓Collagen fibres around dilated congested venules & tubules
Ilbey *et al.*[37]	Testes	*In vivo*/Rat	Cyclophosphamide & 100 mg/kg & po, Cisplatin & 7 mg/kg & ip	10 mg/kg & ip	↓Body & testicular weights, ↓Sperm count & motility, ↑Abnormal sperm, ↓Testosterone Level, ↓GSH-Px activity & GSH level, ↑MDA, ↓Germ cells & irregular seminiferous tubules, ↓Seminiferous epithelial layers, ↓Spermatogenesis & Johnsen score	↑Body & testicular weights, ↑Sperm count & motility, ↑Testosterone Level, ↑GSH-Px activity & GSH level, ↓MDA, ↑Spermatogenesis, Improve testicular morphologic characteristics & perivascular fibrosis & hyalinization of intertubular
Ateşşahin *et al.*[43]	Testes	*In vivo*/Rat	Cisplatin & 7 mg/kg & ip	10 mg/kg & ip	↓Testes & epididymis & seminal vesicle & prostate weights, ↓Epididymal sperm concentration, ↓Sperm motility, ↑Abnormal sperm, ↑MDA & GSH levels, ↓GSH-Px activity. ↑Degeneration & Necrosis & interstitial oedema, ↓Diameter of STs & germinal cell thickness, ↑Desquamative germinal in ST lumen & syncytial cell & severe atrophy	↑Testes & epididymis & prostate weights, ↑Sperm concentration, ↓Abnormal sperm rates, ↑Sperm motility, ↓MDA & GSH levels, ↑GSH - Px activity, ↑Germinal cell thickness, ↓Desquamative germinal cells & severe atrophy

Table 1 (*Continued*)

Author' names	Type of reproductive injury	Models	Chemical & dosage & route of administration	Melatonin dosage & route of administration	Chemical outcomes	Melatonin co-treatment outcomes
Madhu *et al.*[44]	Testes	*In vivo*/Rat	Cisplatin & 3 mg/kg & ip, Vinblastine & 0.15 mg/kg & ip, Bleomycin & 0.5 mg/kg & ip	10 mg/kg & ip	↓Testes weights & size, ↓Spermatogenesis, ↓Activity of 3b- & 17b-hydroxysteroid dehydrogenases, ↓Serum testosterone, ↑Serum FSH & LH levels, ↑LP levels, ↓SOD & CAT activity, ↓Seminiferous tubule size, ↑Inter tubular spaces, ↓Seminiferous tubular diameter & epithelial height, ↓Seminiferous tubular area	↑Testes weights & size, ↑Spermatogenesis, ↑Activity of 3b- & 17b-hydroxysteroid dehydrogenases, ↑Serum testosterone, ↓Serum FSH & LH levels, ↓LP levels, ↑SOD & CAT activity, ↑Seminiferous tubule size, ↓inter tubular spaces, ↑Seminiferous tubular diameter & epithelial height, ↑Seminiferous tubular area
Lee *et al.*[49]	Testes	*In vivo*/Rat	Doxorubicin & 10 mg/kg & ip	15 mg/kg & ip	↓Body & seminal vesicles weight, ↓Sperm count & motility, ↓Spermatogenesis, ↓GR & GST & SOD & CAT activates, ↓MDA & GSH level	↑Sperm count & motility, ↓Incidence & severity of histopathological lesions, ↓MDA level, ↑GST & GR & SOD & CAT activates
Patil *et al.*[48]	Testes	*In vivo*/Rat	Doxorubicin & 3 mg/kg & ip	6 mg/kg & po	↓Body & testes weight, ↓Sperm count, ↑Serum CK & LDH & GOT, ↓Serum testosterone, ↑LPO, ↓GSH & SOD & CAT levels, ↓Na^+K^+ATPase, Ca^{2+}ATPase & Mg^{2+}ATPase, ↑Vacuolization & fibrinoid debris, ↓germ cell in seminiferous tubules, ↑Widening of the interstitial space & vacuolization in interstitial tissues	↑Body & testes weight, ↑Sperm count, ↓Serum CK & LDH & GOT, ↑Serum testosterone, ↓LPO, ↑GSH & SOD & CAT levels, ↑Na^+K^+ATPase & Ca^{2+}ATPase & Mg^{2+}ATPase, ↓Vacuolization & fibrinoid debris & ↑Germ cell in seminiferous tubules, ↓Widening of the interstitial space & vacuolization in interstitial tissues
Alp *et al.*[51]	Testes	*In vivo*/Rat	Procarbazine & 62.5 mg/kg per week & po	10 mg/kg & ip	↓Testicle width & length, ↓Spermatogenesis & Sertoli cells & seminiferous tubule & germinative layer thickness, ↑MDA & ↓ GSH-Px levels,↑NO2/NO3	↓MDA & ↑GSH-Px levels, ↓NO_2/NO_3
Mirhoseini *et al.*[53]	Testes	*In vivo*/Mice	Busulfan & 40 mg/kg & ip	20 mg/kg & ip	↓Spermatogenesis, Destroy in testis tissue, ↓Johnson score, Disruption in seminiferous tubules & germinal cells, ↑Vacuoles in epithelium, ↓Thickness of seminiferous tubules, ↑Apoptosis	↑Spermatogenesis, ↓Johnson score, ↓Damage of germinal cells, ↓Epithelium height, ↓Degeneration of the seminiferous tubules & germinal cells

Table 1 (*Continued*)

Author' names	Type of reproductive injury	Models	Chemical & dosage & route of administration	Melatonin dosage & route of administration	Chemical outcomes	Melatonin co-treatment outcomes
El Aziz *et al.*[64]	Testes	*In vivo*/Rat	Busulfan & 20 mg/kg & ip	10 mg/kg & ip	↑Atrophied & irregular shape in seminiferous tubules, ↓Spermatogenesis & germ cell layers, ↑Multinucleated giant cells, ↑Caspase-3 activity, ↓Positive-PCNA in germinal cells	↑Normal seminiferous tubules, ↑Spermatogenesis & germ cell, ↓Caspase-3-activity, ↑Positive-PCNA in germinal cells
Ghasemi *et al.*[55]	Testes	*In vivo*/Rat	Busulfan & 20 mg/kg & ip	10 mg/kg & ip	↓Quality & quantity of spermatogenesis, ↑Abnormal sperm, ↑Degeneration of seminiferous tubules & desquamative of germinal cells, ↓Johnsen's score, ↑Vacuoles inside in germinal epithelium, ↓Sertoli cells, ↓Serum testosterone level, ↑Apoptosis, ↓BrdU index of germ cells	↑Quality & quantity of spermatogenesis, ↓Abnormal sperm, ↑Johnsen's score, ↑Serum testosterone level, ↓Apoptosis, ↑BrdU index of germ cells
Li *et al.*[56]	Testes	*In vivo*/Mice	Busulfan & 30 mg/kg & ip	10 mg/kg & ip	↓Size & weight, ↓Spermatogenesis, ↓PLZF & SOX9, ↑Apoptosis, ↑caspase-3 & MnSOD & SIRT1 expression, ↓P53, ↑ERK & p38 Phosphorylation, ↑ROS, ↑Mitochondrial dysfunction	↓ROS, ↓Mitochondrial dysfunction, ↑Spermatogenesis, ↑MnSOD & SIRT1 expression, ↓P53 acetylation
Jang *et al.*[45]	Ovary	*In vivo*/Mice	Cisplatin & 2 mg/kg & ip	15 & 30 mg/kg & ip	↓Body & ovary sizes, ↑Follicles activity, ↑Abnormal multifolliculogenesis & primary follicles, ↓Primordial follicles, ↑Activated primary follicles, ↑Apoptosis, ↑PTEN & AKT & GSK3β & FOXO3a & ERK phosphorylation	↑Body & ovary sizes, ↓Primary follicle formation, ↑Dormant primordial follicle, ↓Follicles activity, ↓Apoptosis, ↓PTEN & AKT & GSK3β & FOXO3a & ERK phosphorylation
Pazhuhi *et al.*[57]	Ovary	*In vivo*/Mice	Busulfan & 20 mg/kg & ip	10 mg/kg & ip	↓Ovarian follicles, ↑Mature atrophied follicles, ↓Nucleus size	↓Mature atrophied follicles
Kim *et al.*[61]	Ovarian carcinoma cells	*In vitro*/SK-OV-3 cells	Cisplatin & 80 μm	0, 0.5, 1, & 2 μM	↓Cell viability by 20%, ↑Apoptosis & caspase-3 activity & cleavage of PARP, ↓Phosphorylation of ERK & p90RSK, ↑Dephosphorylation of HSP27	↓Synergistically cell viability dose dependently, ↑Synergistically apoptosis & activation of caspase-3 & cleavage of PARP, ↓Synergistically phosphorylation of ERK & p90RSK, ↑Synergistically dephosphorylation of HSP27, ↑MKP3 level

Table 1 (*Continued*)

Author' names	Type of reproductive injury	Models	Chemical & dosage & route of administration	Melatonin dosage & route of administration	Chemical outcomes	Melatonin co-treatment outcomes
Pariente et al.[60]	Human cervical cancer	*In vitro*/HeLa cells	Cisplatin & 20 μm	1 mM	↓Cells viability & caspase-3 & 9 activation, ↑Early apoptosis, ↑Late apoptosis, ↑ROS	↓Synergistically cells viability & caspase-3 & 9 activation, ↑Synergistically early apoptosis & late apoptosis & ROS
			5-fluorouracil & 1 mM	1 mM	↓Cells viability, ↑Caspase-3 & 9 activation, ↑Early apoptosis, ↑Late apoptosis, ↑ROS	↓Synergistically cells viability & caspase-3 & 9 activation, ↑Synergistically early apoptosis & late apoptosis, ↑ROS
			Doxorubicin & 20 μm	1 mM	↓Cells viability, ↑Caspase-3 activation	↓Synergistically cells viability & caspase-3 activation

↑, increase; ↓, decease; &, and; TBARS, thiobarbituric acid reactive substances; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; GR, glutathione reductase; GST, glutathione S-transferase; CK, creatine kinase; LDH, lactate dehydrogenase; GOT, glutamic oxaloacetate transaminase; LPO, lipid peroxidation; GSH-Px, glutathione peroxidase; FSH, follicle stimulating hormone; LH, luteinizing hormone; PCNA, proliferating cell nuclear antigen; ERK, extracellular signal-regulated kinase; p90RSK, phospho-90-kDa ribosomal S6 kinase; PARP, poly ADP ribose polymerase; HSP27, heat-shock protein 27; PLZF, the promyelocytic leukaemia zinc finger; SOX9, SRY-related HMG-box 9; MnSOD, manganese superoxide dismutase; SIRT1, Sirtuin type 1; p38, p38 mitogen-activated protein kinase; ST, seminiferous tubule; PTEN, phosphatase and tensin homolog; AKT, v-akt murine thymoma viral oncogene 1; GSK3β, glycogen synthase kinase 3 β; FOXO3a, forkhead box o3; BrdU, bromodeoxyuridine.

growing follicles and melatonin attenuated apoptosis.[45,46] Moreover, cisplatin upregulated phosphorylation of PTEN and FOXO3a.[45]

Doxorubicin (or Adriamycin) belongs to anthracycline family and were firstly isolated from *Streptomyces peucetius*.[46] Doxorubicin is mostly used to cure cancers including stomach, lung, breast, bladder, thyroid, soft tissue sarcoma, Hodgkin's lymphoma, multiple myeloma and mainly in ovaries.[47] Doxorubicin significantly increased oxidative stress markers such as LPO, MDA and GSH levels but decreased glutathione S-transferase (GST), glutathione reductase (GR), CAT and SOD activity.[48] Melatonin with its antioxidant property normalized level of MDA and activity of GST, GR and SOD as well as GSH content. Also, CAT activity was slightly higher in the doxorubicin in combination with melatonin group compared to the doxorubicin group.[48] Furthermore, doxorubicin caused an elevation in serum levels of glutamic oxaloacetate transaminase, creatine kinase and lactate dehydrogenase, which is noticeably reduced by melatonin.[48] Doxorubicin also significantly decreased weights of body, testes and seminal vesicles.[48,49] Co-administration of melatonin slightly improved body weight.[49] In another study, co-administration of melatonin with doxorubicin restored body and testes weight compared to normal group.[48] Doxorubicin caused a lot of injuries in testes tissue, including decrease in spermatogonia and early spermatocytes, as well as vacuolation and fibrinoid debris; however, melatonin restored these changes.[48,49] Doxorubicin significantly leads to reducing the levels of membrane-bound enzymes like Ca^{2+}ATPase, Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase. It is shown that co-administration of melatonin significantly normalized these enzymes.[48]

Procarbazine is an anticancer drug used in the treatment of Hodgkin's lymphomas, multiple myeloma, brain tumour, melanoma and lung cancer.[50] In the testes, procarbazine caused a decrease in spermatogonia levels and germinative layer diameter. These criteria were increased by melatonin.[51] Also, morphology, localization and maturation of germinative cells and Sertoli cells were improved by melatonin group vs procarbazine group.[51] Procarbazine significantly changed oxidative stress markers such as increasing levels of MDA and nitrite/nitrate (NO_2^-/NO_3^-) as well as decreasing activity of GSH-Px. On the other hand, activity of GSH-Px is reversed by melatonin.[51]

Busulfan (butane-1,4-diyl dimethanesulfonate) is an alkylating drug and used as an alleviating management in malignant diseases, including polycythemia vera and myeloproliferative disorders.[52] Busulfan is a very toxic chemotherapy drug, which causes side effects in multiple organs, including the reproductive system.[53,54] Busulfan

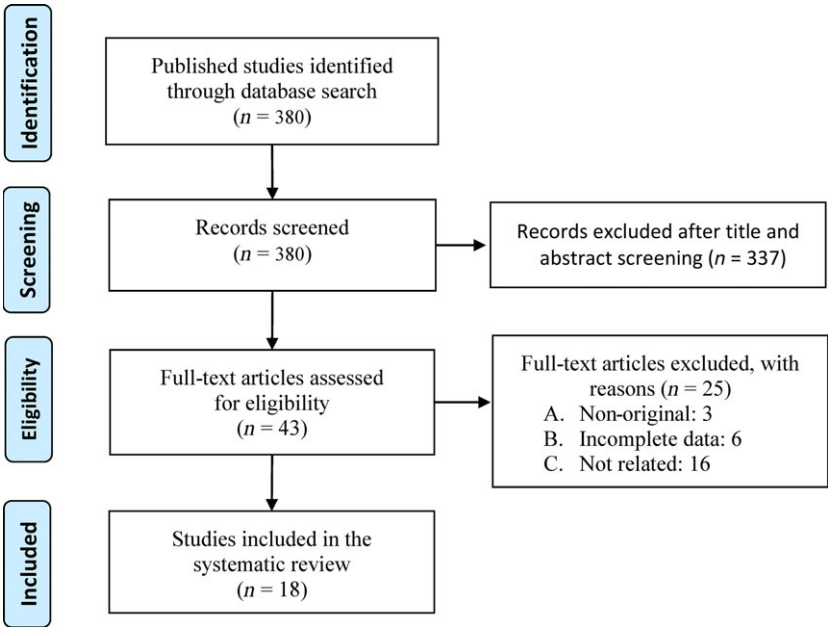

Figure 1 Flow diagram of the study selection process. [Colour figure can be viewed at wileyonlinelibrary.com]

induced disruption in seminiferous tubules structure, degeneration in germinal cells and a significant decrease in thickness of the seminiferous tubules in the testis.[53,55] Melatonin co-administration attenuated these consequences with its protective properties. Moreover, busulfan reduced epididymal sperm motility and concentration and also increased abnormal sperm rates.[55] Melatonin co-administration increased sperm count and motility and decreased rates of abnormal sperm. In addition, busulfan increased apoptosis in germ cells and seminiferous tubules.[53,55,56] On the other hand, melatonin co-administration either significantly or non-significantly decreased apoptosis in seminiferous tubules and germ cells.[53,55,56] In addition, busulfan remarkably decreased testosterone levels, which is normalized by melatonin.[55] Busulfan causes oxidative stress via decreasing manganese superoxide dismutase (MnSOD) expression and increasing intracellular ROS, which is modulated by melatonin. Furthermore, busulfan induced mitochondrial dysfunction. However, melatonin co-administration partially decreased mitochondrial dysfunction.[56] Besides that, busulfan induced apoptosis, consequently spermatogonia loss via upregulation of caspase-3, downregulation of Sirtuin type 1 (SIRT1) and increased extracellular signal-regulated kinase (ERK) as well as p38 phosphorylation.[56] Co-administration of melatonin with busulfan significantly increased SIRT1.[56] Busulfan significantly destroyed ovarian follicles and mature atrophied follicles while melatonin co-administration noticeably decreased mature atrophied follicles.[57]

Effects of melatonin on sexual hormones and receptors-mediated from toxicity of chemotherapeutic drugs

Sexual hormones are related to sexual function, vitality and physical function. Numerous reports have evidenced that the chemotherapeutic drugs induce an alteration in sexual hormones level. Cisplatin alone or in combination with vinblastine, bleomycin, cyclophosphamide, doxorubicin and procarbazine significantly reduced plasma level of testosterone, whereas melatonin co-administration significantly increased plasma testosterone level.[37,48,51] Moreover, cisplatin in combination with vinblastine and bleomycin increased FSH and LH levels, while normalized by melatonin.[44]

Chemosensitizing effects of melatonin from toxicity of chemotherapeutic drugs

5-fluorouracil (5-FU) is an antimetabolite chemotropic drug acts through two mechanisms including interfering with DNA and RNA that results in production of macromolecules as well as inhibiting essential biosynthetic functions.[58] 5-FU is widely used in the treatment of cancers, including breast, colorectal, head and neck and aerodigestive tract ones.[59] In a study, 5-FU significantly decreased human cervical carcinoma (HeLa) cells viability compared to the control group. Interestingly, co-administration of 5-FU with melatonin significantly increased 5-FU stimulated cytotoxicity. Furthermore, treatment of HeLa cells

with 5-FU and melatonin elevated caspase-3 and caspase-9 activity as well as ROS generation compared to the 5-FU group.[43]

Cisplatin decreased the viability of SK-OV-3 ovarian cancer cells by 20%, and melatonin dose dependently increased the viability of cells.[60] Cisplatin also increased apoptosis in SK-OV-3 cells, whereas melatonin in combination with cisplatin synergistically increased apoptosis.[61] In this regard, melatonin increased activation of caspase-3 and cleavage of Poly-(ADP ribose) polymerase (PARP) induced by cisplatin.[61] It is also shown that cisplatin prevents phosphorylation of heat-shock protein 27 (HSP27), ERK and phospho-90-kDa ribosomal S6 kinase (p90RSK), whereas in synergistic manner melatonin with cisplatin decreased phosphorylation of HSP27, ERK and p90RSK compared to cisplatin group.[61] Cisplatin alone or in combination with melatonin inhibited the expression and colocalization of HSP27 and p90RSK in SK-OV-3 cells compared to the control or melatonin groups.[61] Furthermore, melatonin or cisplatin had no significant effect on mitogen-activated protein kinase phosphatase 3 (MKP3) levels but cisplatin in combination with melatonin significantly increased MKP3 level.[61] In another study, melatonin sensitized HeLa cells to cisplatin and doxorubicin toxicity. Cisplatin and doxorubicin reduced HeLa cells viability compared to the control group and interestingly co-administration of melatonin significantly reduced viability. Moreover, cisplatin increased caspase-3 and 9 activity, ROS generation and eventually apoptosis and melatonin in combination with cisplatin elevated ROS generation, caspase-3 and 9 activity as well as apoptosis compared to the cisplatin group.[60] Doxorubicin induced increase in caspase-3 activity and melatonin in synergism manner increased caspase-3 activity.[61]

Discussion

In this review, the impairments induced by cyclophosphamide, cisplatin, doxorubicin, procarbazine, busulfan and 5-FU drugs on the reproductive system, as well as co-administration effects of melatonin on these disorders were evaluated. Furthermore, the chemosensitizing effect of melatonin on SK-OV-3 and HeLa cells in concurrent treatment with cisplatin was assessed. The important effects of melatonin in normal and cancer cells are illustrated in Figures 2 and 3.

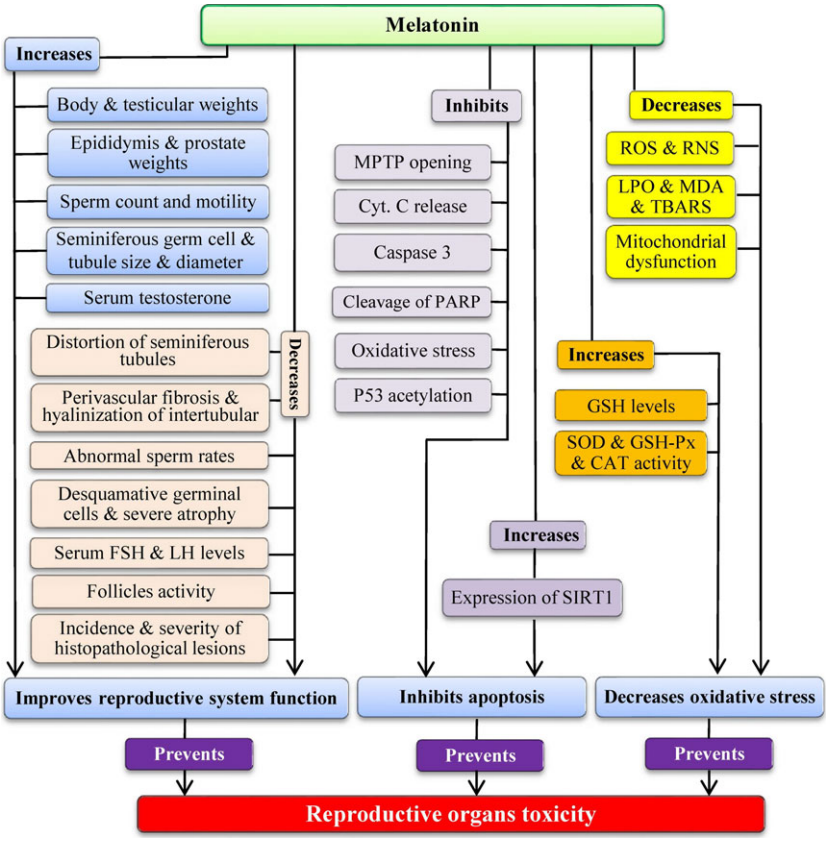

Figure 2 Important effects of melatonin in normal cells. [Colour figure can be viewed at wileyonlinelibrary.com]

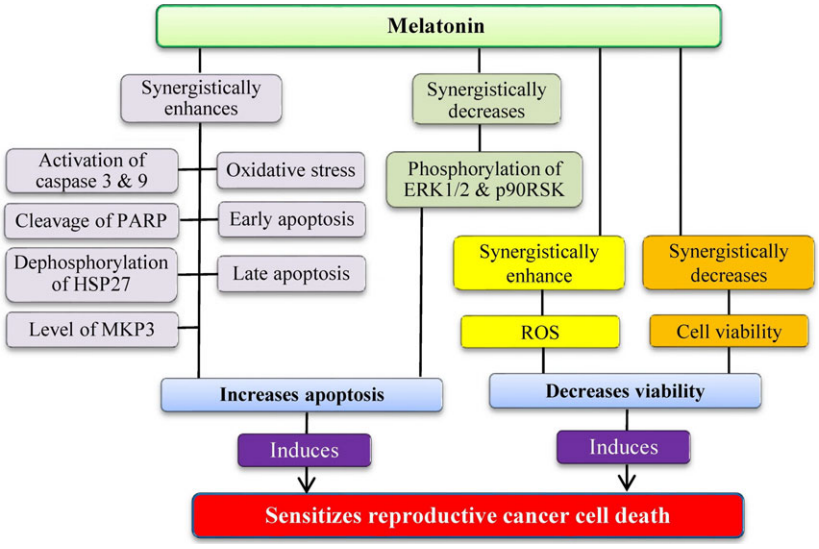

Figure 3 Important effects of melatonin in cancerous cells. [Colour figure can be viewed at wileyonlinelibrary.com]

Although chemotherapeutic regimes are highly effective in the treatment of different malignancies, their clinical utility is limited because of a wide range of side effects such as nausea, vomiting, dysphoria, injury to kidney, lung, reproductive organs. The reproductive system includes some of the external and internal organs that participate together for the purpose such as vitality, physiological function and procreating. Because of its critical function in the survival of the species, it has attracted the attention of many researchers. These organs are extremely vulnerable to chemotherapy drugs, because of their structures. The toxicity of these drugs in reproductive systems is associated with variable factors, such as the number of chemotherapeutic drugs, antineoplastic drug group, treatment duration, their total doses and individual sensitivity.[62,63] Using protective agents during chemotherapy has been proposed as a possible solution to reduce chemotherapeutic side effects. Co-administration of melatonin during chemotherapy acts as a double-edged sword on tumoral and normal organs/cells. In the other words, in normal organs/cells, melatonin results in the preservation of testicular epididymis, Sertoli cell, testes and prostate weights, improve sperm count and modulates sexual hormone levels through their antioxidant, anti-apoptotic, antiproliferative effects, etc. Furthermore, it has chemosensitizing effects on tumoral cells through increasing oxidative stress, apoptosis, etc.[32,37,39,43,48,49,51,53,55,64] In the following paragraphs, various mechanisms of melatonin on the reproductive system are discussed.

Antioxidant effects of melatonin

Oxidative stress is an imbalance between the biological defences and generation of free radical agents. Increasing the intracellular level of free radicals, as an oxidative stress biomarker, indicates toxicity potential, which reacts with cell macromolecules and cell dysfunctions.[65] The chief property of antioxidants is the capacity to capture free radicals. Recently, the role of melatonin as a high potent antioxidant has attracted many attentions. The primary protection mechanisms of melatonin seem to be undertaken through both direct and indirect pathways.[66,67] Melatonin in direct way scavenges the singlet oxygen, peroxyl radical, superoxide anion radical and peroxynitrite anion.[68–70] Each melatonin molecule scavenges two hydroxyls-free radical ($^{\cdot}OH$).[70] In addition, melatonin is capable of increasing the efficiency of mitochondrial electron transport and decreasing free radical generation and electron leakage. Therefore, it is concluded that the capability of melatonin as a direct free radical scavenger may be due to its electron-donating potency.[71] Unlike well-known antioxidants like vitamin E and C, which are exclusively lipid and water-soluble respectively, melatonin has a limited intracellular distribution. Because of the small size and amphiphilic property melatonin can transit from cell membrane easily so that it preserves macromolecules against free radical induced injury in both aqueous subcellular and lipid segments.[43,72] Moreover, it has been reported that melatonin is five times more impressive in scavenging hydroxyl radicals than glutathione.[73] In addition, melatonin metabolites have antioxidant effects too, even more than their ancestor. Melatonin can decrease non-enzymatic LPO in rat testicular mitochondria and microsomes by changing both docosapentaenoic and arachidonic acids.[74] It has been reported that the restoration of membrane-bound enzymes like Mg^{2+} ATPase, Ca^{2+} ATPase and Na^{+} K^{+} ATPase in rats treated with melatonin

is due to the membrane stabilizing related to the protective effect of melatonin.[75] Furthermore, it was shown that melatonin can break the vicious cycle of ROS generation and mitochondrial damage. In other words, it has an indirect stimulatory effect on antioxidant enzymes including GR, SOD, GSH-Px and CAT.[76–80] Additionally, melatonin enhances MnSOD expression, which causes a reduction in ROS concentration.[56] Melatonin was recognized as an inhibitor of the ubiquitin-proteasome system.[81] Melatonin modulates its non-enzymatic and enzymatic antioxidants property due to modification of antioxidant enzyme gene expression and inactivation of nuclear ROR-alpha melatonin receptor through reaction with calmodulin.[51] Furthermore, melatonin with its strong antioxidant effects on ovarian follicles has effects in growing fetus, maturating oocyte, reducing the oxidative stress during ovulation, decreasing number of mature atrophied follicles and preserving structures such as theca follicular and zonapellucida.[57]

Anti-apoptotic effects of melatonin

Apoptosis is a regulated form of cell death which is necessary for survival and homoeostasis in multicellular life forms. Physiologically, apoptosis decreases transformed or harmed cells and requires to control cell numbers, organ and tissue morphology. Any irregularity in apoptosis process often results in cancer and tissue disorders.[43] Melatonin supplementation promotes the efficiency of murine-induced pluripotent stem cells production via the inhibition of p53 in apoptosis pathway.[82] As a result, p53 is a significant protein related to the effects of melatonin and busulfan. However, melatonin reduces the concentration of p53 because of enhancing the SIRT1 expression.[56] Melatonin improved histopathological changes and apoptosis via the unfolded protein response in testes and inhibition of endoplasmic reticulum stress.[83,84] Nuclear FOXO3a is needed for keeping quiescence of the primordial follicles. After phosphorylation of FOXO3a and PTEN, they are translocated to the cytoplasm during follicle activation. Cisplatin or melatonin did not affect FOXO3a expression. However, cisplatin can induce an increase in FOXO3a and PTEN phosphorylation. Melatonin downregulates phosphorylation of FOXO3a and PTEN.[45] Thus, melatonin reduced cisplatin-induced exhaustion of primordial follicle in the ovary mainly via phosphorylation and activation of PTEN/AKT/FOXO3a pathway.[45]

Antiproliferative effects of melatonin

It has been reported that the increment of proliferating cell nuclear antigen in testicular germ cells demonstrate high proliferative and stimulation of spermatogenesis.[85] It seems that mitotic proliferation of germ cells is dependent on the c-kit system, stem cell factor,[86] GnRH[86–89] and estradiol concentrations.[90,91] Furthermore, melatonin can decrease the damage effects of busulfan through the two procedures. Firstly, it reduces the release of LH and FSH via the direct effect on hypothalamic–pituitary–gonadal axis and secondly, it has a positive effect through an indirect antiproliferative effect on the germ cells and other cells. In a study by Hermann *et al.*,[92] it is reported that melatonin has antiproliferative effects on mouse hepatoma cell line. In another study, Bartsch *et al.*[93] indicated that melatonin has an inhibitory effect on ovarian carcinoma in individual patients.

Effects of melatonin on sexual hormones and receptor-mediated

Melatonin and the sex hormones are interrelated under both pathological and physiological conditions. Chemotherapeutic drugs can alter sexual hormone levels and co-administration of melatonin effects these alterations. The increment of testosterone level is consistent with protection effect of melatonin on Leydig cells; because of existence of melatonin binding sites in the reproductive system.[55] It also may be due to the direct effect of melatonin on the steroidogenic cells of the reproductive systems.[94] In another study, it has been shown that increasing testosterone levels, due to melatonin effect on Leydig cells, maybe because of the coupling melatonin receptors via pertussis toxin-sensitive G-protein in Leydig cells.[95] Interestingly, there is an inverse relationship between GnRH receptors and melatonin in mice, thus, it directly controls the ovarian activity and reduces secretion of LH and FSH.[96] Additionally, melatonin can alter the function of the hypothalamic–pituitary–gonadal axis via its ability to change the secretion of GnRH through its binding sites in the suprachiasmatic nucleus, hypothalamic premammillary or mediobasal hypothalamus region.[97]

Besides binding sites of melatonin recognized in the various parts of the reproductive organs, it can assume that melatonin also handles its functions not only as an antioxidant but also via direct reaction with its receptors on the steroidogenic cells.[94] According to melatonin receptors detected on the epididymis and low affinity in binding sites of melatonin identified in spermatozoa, it is feasible that melatonin impresses sperm mobility as they pass via the epididymis.[98]

Chemosensitizing effects of melatonin

It has been shown that melatonin meaningfully enhanced the cytotoxic effect of some chemotherapy drugs. For

instance, in a study, melatonin synergistically prevented cisplatin-induced apoptosis through the phosphorylation of ERK association with HSP27 and dephosphorylation p90RSK in SK-OV-3 cells.[61] In addition, melatonin significantly blocked the colocalization and expression of HSP27 and p90RSK by combination treatment with cisplatin.[61] As a result, they suggested that melatonin can be useful in combination with cisplatin to ameliorate the therapeutic effect of cisplatin for treatment of ovarian cancer by targeting the ERK/p90RSK/HSP27 pathway. Moreover, melatonin with cisplatin meaningfully enhanced the ratio of cells going in mitochondrial apoptosis because of ROS overproduction and substantially augmented DNA fragmentation in comparison with cisplatin group in HeLa cells.[60] Recently, several studies showed that melatonin may have a pro-oxidant effect through endoperoxides molecules arisen or NF-kB activation in tumour cells.[99–101] As a result, synergistic antitumour functions related to melatonin are still debatable. It may be dependent on the tissue where malignant cells arose from it and the applied chemotherapeutic drugs.[60]

However, melatonin may reduce the efficacy of chemotherapy based on several chemotherapeutic drugs, many studies have demonstrated that non-protein thiols and intracellular glutathione increased by melatonin due to suppressing platinum, which may lead to resistance to cisplatin chemotherapy.[102,103] Also, Ilbey *et al.*[37] observed that rats receiving cisplatin or cyclophosphamide with melatonin had less body weight loss, which is assumed to be as one side effect of these chemotherapy drugs. Also, in a study, co-administration of melatonin and doxorubicin did not alter MDA and GST level and GR as well as SOD activity compared to doxorubicin group.[49] Chen *et al.*[104] reported that melatonin induces apoptosis in germ cells. So, it was concluded that melatonin effect on the cells is dose- and time-dependent.[105] Racial differences can be a factor for difference in the inherent sensitivity of germ cells to the apoptosis. For example, it has been reported that the incidence of spermatid and spermatogonial programed cell death in Chinese males is more than Caucasian males.[106] However, the exact mechanisms of melatonin effect on the germ cells are not known. In a previous study in association with antiproliferative effects of melatonin, it did not effect on hepatoma cell lines of hamster but observed antiproliferative properties on in-vivo condition.[107] Also, Ozdemir *et al.*[108] did not find melatonin's antiproliferative properties on hepatoma cell line of a human. The race of animals, treatment time and the melatonin dose can be effective on the proliferative property of melatonin. In an apoptosis analysis, it has been indicated that melatonin cannot improve busulfan-induced apoptosis. Given that cancer cells have various ROS generation, tolerance and elimination mechanisms compared with normal cells.[56] In association with the chemosensitizing effect of melatonin, it has been reported that melatonin reduces the antitumour effect of cisplatin in human liver carcinoma cells through the adjustment of the balance of apoptotic proteins.[109] Other reports have shown that melatonin does not participate with the cytotoxic effect of chemotherapeutic drugs like daunorubicin, cytarabine and etoposide in various leukaemia cell lines, consisting of MOLT-4, Daudi, Jurkat, HL-60, K562 and CMK.[110] Kim *et al.*[61] showed that melatonin does not have an effect on levels of phosphor p90RSK and ERK1/2 in ovarian cancer cells; as they showed that co-administration cisplatin and melatonin sensitize the inhibition of phospho-ERK1/2 and p90RSK compared with cisplatin alone.

Perspective of future research of melatonin

Cancer is one of a major condition which affects every society in the world. Cancer incident is varying by age, sex, personal and personal life condition, racial and ethnic group, geographic location and other categories.[1,111] In this content, the most common cancer is related to the non-reproductive system. Although the chemotherapy process is a most effective cure for cancer, the side effect is high because of effects on non-selective and harms to normal cells beside cancer cells.[112] The reproductive system because of its' multiorgan and polyunsaturated fatty acids nature as well as gonadotropin hormones are very vulnerable to toxicity induced by chemotropic drugs.[113,114] Therefore, the researchers are trying to find an effective way to reduce harms to the reproductive system. One of the proposed approaches is co-administration with non-chemotropic drugs which reduce harms to normal cell and multiplier effects of these agents in cancer cells. According to our study, melatonin reduces oxidative stress, apoptosis, inflammation and modulates mitochondrial function.[12,14,15,18,19,27] Also, it can block pathway which leads to reducing harms to normal cells during chemotherapy process in reproductive or non-reproductive cancer, and multiplier pathway related to the increased death of cancer cell by chemotropic drugs. These facts support the previous studies that demonstrate the effects of melatonin in reducing side effects of chemotropic drugs in the reproductive system.[32,37,39,43–45,48,49,51,53,55–57,64] Furthermore, melatonin has a sensitizing role the reproductive cancer cells.[60,61] In this content, melatonin presents several direct and indirect properties including antioxidant, anti-inflammatory, anti-apoptotic, mitochondrial protection and antidepressant property, besides its low toxicity. Considering all of these results, we propose that more researches are needed and melatonin is an attractive pharmacological candidate for combination therapy with chemotropic drugs in clinical trials because it reduces harmful effect in the

reproductive system in vulnerable people during chemotherapy process and enhances the effect of chemotropic drugs to kill cancer cells in the reproductive system.

Conclusion

Chemotherapeutic drugs exert harmful effects on the reproductive system through several pathways. In chemotherapy process, oxidative stress, apoptosis, tissue injury, mitochondrial malfunctions and sexual hormones alteration will rise in normal cells of reproductive system which causes fecundity reduction. Furthermore, chemotherapeutic drugs have deleterious effects on reproductive organs, follicles, ovary and sperm quality and quantity as well as sexual hormones. The low toxicity of melatonin consumption even in high dosage and its cytoprotective effects through several mechanisms bring positive effects in the reproductive system. Melatonin directly through free radicals scavenging and indirectly through modulating antioxidant enzymes reduces oxidative stress. Moreover, melatonin with its anti-apoptotic effects, including inhibition of caspase-3 activation, PARP cleavage and modulating mitochondrial function, reduces apoptosis. In addition, melatonin ameliorates reproductive injury through modulating sexual hormones and antiproliferative effect alongside receptors-mediated properties in reproductive organs. In other hand, it is shown that melatonin has sensitizing effects in cancer cells. Melatonin in combination with chemotherapeutic drugs increases oxidative stress. Also, melatonin increases apoptosis through enhancement of caspase-3 and 9 activity, cleavage of PARP and dephosphorylation of PTEN/AKT/FOXO3a pathway. Taking together, melatonin with its bilateral role can protect the reproductive system during chemotherapy process in both normal and cancer cells.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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Authors' contributions

HHA, MHA, BF and NHG performed the literature search and drafted the manuscript. HHA and MHA presented the idea. HHA prepared the figures. MR edited the manuscript. MA supervised the whole study.

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