Review

Nanocarriers for melatonin delivery

Amirreza Ahmadi Jazi[1], Fatemeh Mohammadzadeh[1], Saeed Amirkhanlou[1,2], Zahra Arab Bafrani[1], Seyed Mostafa Mir[1]*

[1]Metabolic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[2] Department of Nephrology, Clinical Research Development Unit (CRDU), Sayad Shirazi Hospital, Golestan University of Medical Sciences, Gorgan, Iran
*Correspondence: mostafamir1987@gmail.com**,** Tel: 09115288358

Running title: Melatonin delivery

Received June 14, 2023; Accepted: December 18, 2023

ABSTRACT

More attention has been drawn to the drug delivery systems to achieve more precise and efficient treatment for patients with less doses of medicines. The use of nanoparticles for drug delivery system has emerged for this purpose. It can enhance the treatment efficiency by use of the drugs more selectively and precisely to deliver them to the targeted organs or tissues. Drug delivery systems can also help to reduce the side effects, especially for the chemotherapeutic agents that have severe toxicity. Melatonin (N-acetyl-5-methoxytriptamine) is a small indolamine molecule that is produced by most cells and can influence on circadian manner. Melatonin also has antiapoptotic and antioxidant actions depending on the microenvironment; these actions are enhanced when it is incorporated into nanocarriers. Although the therapeutic effects of melatonin are promising, to achieve its optimal results is required. Therefore, the use of nanocarriers of melatonin is of clinical interest. Different melatonin loaded nanocarriers such as lipid-based nanocarriers, hybrid nanocarriers, synthetic ones, etc. can be used to deliver melatonin more efficiently for prevention or treatment of various diseases. In this review, we summarize the treatment efficiency of melatonin when it is incorporated into different nanocarriers.

Key words: Melatonin, nanocarrier, nanoparticle, drug delivery system

---

1. INTRODUCTION

Nanocarriers (NC) are structures usually with two or three dimensions with sizes ranging from 1 to 100 nanometers (1). In some cases nanoparticles smaller than 1000 nanometers are still considered as nanocarriers (2). These structures consist of 3 layers: the surface layer which can have a variety of functionalized groups in order to achieve better interactions and be more useful in the delivery system, the shell layer, and the core which is the main component. There are also liquid crystalline nanoparticles that consist of these 3 mentioned layers dispersed in liquid crystals. The self-assembly of nanomaterials into ordered, yet fluid liquid crystalline superstructures allows us to combine unique properties of nanomaterials with the mobility and ordering of the liquid crystalline state (3, 4). Scientists use these NCs in different fields such as biological sensing, gas sensing, etc. but the most important application of NCs in medicine is in drug delivery systems (5).

Melatonin is methoxy indolamine that is biosynthesized from tryptophan and is secreted by the pineal gland during the darkness to regulate circadian rhythm in vertebrates. This molecule is also produced in many other cells but is not released into the blood; therefore, this locally produced melatonin does not participate in circadian regulation (5, 6). Melatonin has a variety of important actions; it regulates the immune system and has antioxidant and cytoprotective effects (7-10). Melatonin reduces blood pressure and exhibits cardioprotective effects. Also, melatonin is involved in the regulation of hematopoiesis [7] and has antiviral and anti-bacterial actions, as well as protects the liver from oxidative stress (11). Melatonin has short half-life in the blood, low water solubility (5). Melatonin has receptors on cell membranes (MT1, MT2) and in the cytosol (MT3) and some of its actions are mediated by these receptors. Some functions of melatonin are receptor-independent by interacting directly with intra or extracellular molecules (9, 12).

Several routes have been used for melatonin delivery and each has particular characteristics. For example, transmucosal administration of melatonin causes high plasma concentrations compared to melatonin orally. Intranasal instillation exhibits rapid absorption and high bioavailability compared to other routes (13). Transdermal use of melatonin results in different absorption rates because of variation in the thickness of the skin and variable retaining rates in some skin cells. It has been shown that the continuously released melatonin from NC may have greater effectiveness than other methods and it has been proven safe (14). The mechanisms are that the nanostructures provide protection against chemical oxidation of melatonin and improve its penetration, therefore, loading melatonin in nanocarriers exhibits better efficacy (5).

Melatonin has high stability but low solubility in aqueous solutions and is also stable at concentrations of 100 to 113 µg/ml in solution containing 5% ethanol and 95% normal saline for six months. Melatonin at dose of 50µg/ml in phosphate buffer degraded in 21 days. The 10 mg melatonin in 1 ml glycofurol solution (20% w/w) and 40% w/w DMSO was stable at 25°C for 45 days. However, due to the cytotoxicity of these solutes , it is not recommended to store melatonin in those solutions (15). Since melatonin has low solubility and high stability in aqueous environments some modifications can improve its low solubility. Modification of the acetamide component, the substitution of the second position in indole ring, and the substitution of methoxy group have been done to increase its solubility. Research has shown that small N-substituted analogs of melatonin can act as an agonist in contrast to numerous N-substitutions (11). A novel derivative of melatonin, sodium 4-(3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl) butane-1-sulfonate (MLTBS), is synthesized by treating melatonin with NaH and 1,-4 butane sulfone in tetrahydrofuran; it has good solubility in aqueous solutions and high safety in addition to mimicking melatonin's functions (5). To improve bioavailability, solubility and permeability nanocarriers may be the best choice for these purposes. In this review we focus on the nanocarriers which are used in order to improve melatonin's efficacy (Figure1).

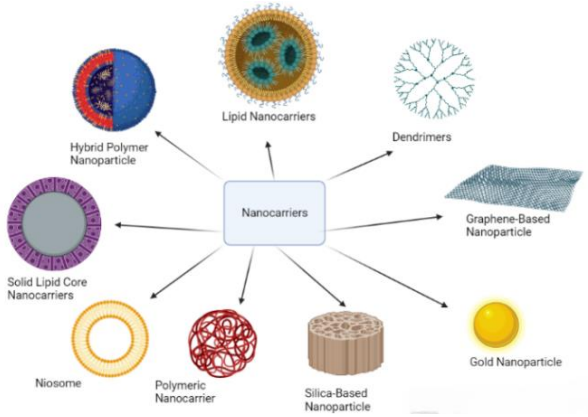

Fig.1. melatonin loaded nanocarriers which are summarized in this review.

2. LIPID-BASED NANOCARRIERS

2.1. Liposomes.

Liposomes contain an aqueous core surrounded by a phospholipid bilayer. The potential to carry numerous molecules (hydrophilic substances in the core, hydrophobic substances in the membrane bilayer, and amphiphilic substances within the interface of lipid and aqueous parts) has made them popular. Techniques used to synthesize liposomes are those of reverse phase evaporation, ethanol injection, homogenization at high pressure, pH gradient methods, and detergent dialysis (16).

Liposomes have low loading capacity and stability and they are also expensive. An *in vitro* study showed that melatonin-loaded liposomes had slower release rate than 10% ethanol dissolved one (17). Recent studies show that zwitterionic polymers stabilizes liposomes (18). Gonçalves *et al*. (19) used chitosan to enhance the stability of phosphatidylcholine liposomes loaded with melatonin as chitosomes. It improved the stability of melatonin at room temperature and decreased the diameter of the membrane's bilayer which enhanced the release of melatonin. Zhang *et al*. (16) found under the pressure of CO_2 (140 bar), melatonin-loaded liposomes reached a better encapsulation efficiency (EE = 82.2%) with small average diameter of 66 nm which provides optimal bioavailability for melatonin absorption. Melatonin-loaded liposomes containing ethanol (ethosomes) are suitable for its transdermal delivery (20). To improve the penetration of melatonin to the skin, Marepally *et al*. (21) produced a new lipid melatonin delivery system, i.e., the formulation 1, 1-Di-((Z)-octadec-9-en-1-yl) pyrrolidin-1-ium iodide (Cy5), a most efficient lipid to enhance the penetration (134 µg/cm^2 after 5 h). In a study, melatonin-loaded liposomes at dose of 4.46 mg/kg significantly inhibited the oxidative damage in the livers and lungs of rats (22). The melatonin-loaded nanocapsules prepared by phase inversion method (23) was also investigated for its ischemic neuroprotective effect. These nanoparticles showed good storage stability and a sustained release after a burst drug release and higher permeation capacity. The particles sizes were from 18.26 to 109.8 nm. The post ischemic administration of Mel-LNC intranasally lowered oxidative stress by decreasing NO, myeloperoxidase and malondialdehyde levels and increased superoxide dismutase activity and glutathione level. Hou *et al*. (24) prepared melatonin-loaded elastic liposomes with sodium deoxycholate (liposomes that contain a biocompatible membrane softener such as sodium cholate, twin, and span) for percutaneous melatonin delivery. The method was thin-film dispersion with a high encapsulation efficiency (73.91%). The results showed that melatonin-loaded elastic liposomes (Mel-EL) had 1.5 times higher penetration capacity and resulted in enhancing the skin hydration level, ameliorating skin elasticity, and preserving the integrity of dermal collagen compared to the control. These researches are summarized in Table 1.

Table 1. The biological effects of melatonin in different liposomal carriers.

Liposomal Carrier	Biological effects
Chitosan phosphatidylcholine	Improved the storage duration and decreased the bilayer thickness (19)
Melatonin loaded liposomes	Inhibit the oxidative damage in liver and lung of rats (22)
Elastic liposomes with sodium Deoxycholate	Higher penetration and enhancing skin hydration and elasticity (24)
Melatonin loaded nanocapsules	Lowered oxidative stress by reduction of NO, Myeloperoxidase and malondialdehyde levels and increase in SOD and glutathione (23)

2.2. Solid lipid nanocarriers (SLN).

These nanoparticles were introduced for first time in late 1991 (25). Solid lipid nanocarriers consist of a solid lipid core that is surrounded by a surfactant layer. The core is solid at room and body temperature and has a melting point above 40°C (5). These carriers have the unique ability to deeply penetrate into organs especially the CNS which hydrophobic barriers.

A new version of SLN called nanostructured lipid carrier was produced from both solid and liquid lipids in order to enhance stability and loading capacity. Nanostructured lipid carriers (NLC) were first developed in the late 1990s as alternatives to solid lipid nanoparticles and to avoid undesired expulsion of cargo molecules from the matrices (26). SLN and NLC are made from the attachment of the drug into melted lipids and then adding them to the aqueous surfactant solution. Different methods are used to develop SLNs and NLCs, for example, high energy methods (ultrasound techniques and homogenization at high pressure) and low energy methods (microemulsion, solvent emulsification evaporation, and coacervation) and both of them are followed by immediate cooling (5). Albertini *et al.* (27) used SLNs that contain 3 to 6 mg melatonin to enhance its bioavailability to treat infants or children with neurodevelopmental difficulties. Musazzi *et al*. (28) produced melatonin-loaded SLN and incorporated it into dispersible films which are made of maltodextrins and achieved a sustained release over at least 5 hours in saliva, in gastric and intestinal environment.

SLN and NLC can be administered locally, orally, systemic, and ocularly. Studies show that free melatonin is degenerates 19.6% during light exposure (at dose of 20 MED which is considered representative of daily solar emission) but when it is incorporated in tristearin-phosphatidylcholine SLN, the degeneration was reduced to 5.6% (29). Hatem *et al*. (30) used melatonin (25 mg) loaded NLC which has polysorbate 80 on its surface as a surfactant to treat androgenic alopecia. Because of the sustained release and better permeability of NLC preparation, patients' hair density was reportedly increased and hair loss was reduced.

In a melatonin-loaded SLN study, cyclosporine A (CsA) (15 mg/kg/day) to was used to cause cardiotoxicity with lipid peroxidation and apoptosis and the melatonin loaded SLN (1 mg/kg/day) showed rapid absorption and thus showed antiapoptotic efficacy compared to the CsA alone group (31). Mirhoseini *et al*. (32) used single dose of melatonin-loaded SLN (25mg/kg) to investigate its antioxidant effect. They observed decreased levels of malondialdehyde in rats' testis and improved the thickness and diameter of the seminiferous epithelium in rats with testicular trauma. Sabzichi *et al.* (33) incorporated melatonin into NLC and then investigated its codelivery via tamoxifen on MCF-7 cell line. In addition to inhibiting cell proliferation, it enhanced the apoptotic effects of tamoxifen with a marked decrease in tumor cell survival and increased levels of Bid mRNA compared to the control. Another study was designed to investigate the role of melatonin-loaded NLC in the *in vitro* fertilization. Parvez *et al*. (34) loaded a combination of 2-hydroxypropyl-β-cyclodextrin (HPβCD) and amphotericin B in SLN and administered it orally for the treatment of visceral leishmaniasis (VL). The formulations were synthesized by the emulsion solvent method and showed a high encapsulation efficiency for both melatonin (63 ± 6.24) and amphotericin B (87.9 ± 0.57). The nanoparticle has lowered the intracellular parasite load significantly (99.98%) and therefore, it would be a good therapeutic agent in the treatment of VL. Aghaz *et al*. (35) evaluated the synergistic effects of melatonin and resveratrol (RES) co-encapsulated by SLN compared to their normal combination (Mel + RES) on mouse germinal vesicle oocytes. Germinal vesicle (GV)-stage oocytes harvested from 6- to 12-week-old female NMRI mice were randomly divided into different concentrations of Mel+RES or Mel+RES-SLN groups. The results showed a significant improvement in the normal morphology of warmed GV-stage oocytes, GV breakdown (GVBD) rate, metaphase II (MII)-stage oocyte formation, fertilization rate, early embryo development, and a significant reduction in intra/extracellular ROS level in

lowest concentration of Mel+RES-SLN group compared to its corresponding Mel+RES group. The results indicated that Mel+RES-SLN could enhance/control intracellular penetration compared to the Mel+RES. They also reported that the highest concentration of Mel+RES-SLN was safe, without a detrimental effect on embryonic development upon treatment. The beneficial effects of melatonin-loaded lipid nanocarriers are summarized in Figure 2.

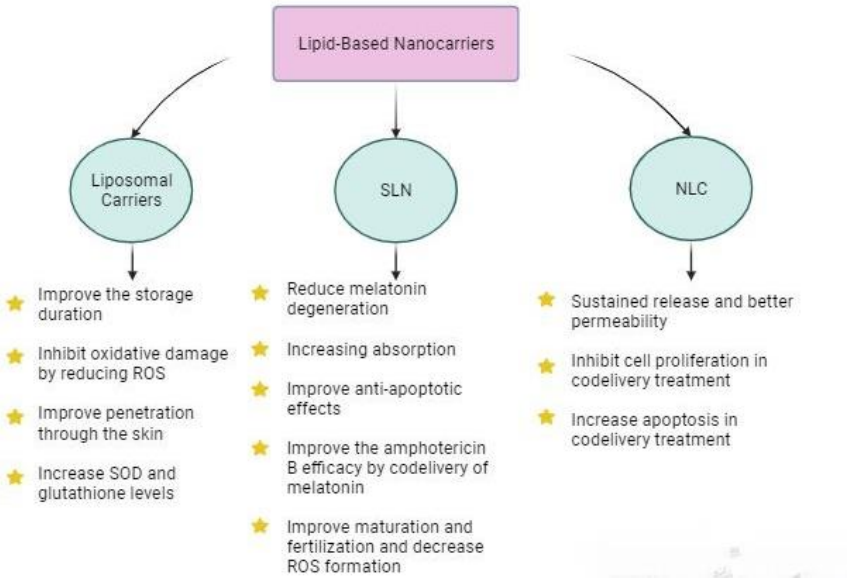

Fig. 2. Review of the roles of melatonin-loaded lipid nanocarriers.

3. HYBRID CARRIERS

Hybrid carriers are those particles that consist of lipid and polymers as carriers of melatonin. The lipid part increases loading capacity and improves drug permeation and the biocompatible polymer regulates the drug release (36). To produce lipid polymer hybrid nanocarriers (LPN), sorbitan monostearate is diffused in capric triglyceride then it is surrounded by polymer and at last it is coated with polysorbate 80 (37). Melatonin at a dose of 400 µM was sufficient to protect against lipid peroxidation. The encapsulation efficacy of LPN (55%) was higher than that of nanoemulsion (33%), so, LPN was more efficiency as an antioxidant carrier than that of nanoemulsion (37). It is suggested to use cationic lipids and polymers on the nanocarriers' surface to enhance the stability and interaction of LPN with biological membrane. Carbone *et al*. (38) compared hybrid nanoparticles with polymeric nanoparticles produced by a low-energy-organic-solvent-free method. They observed that good coating properties clearly influence on physical properties such as shape, size and stability. They also found that dimethyl dioctadecyl ammonium bromide was the most suitable coating material for LPN.

LPN-melatonin could also be used in cell culture which significantly enhanced the efficiency of mammalian embryos and oocyte quality (39). The highest hatching rate was observed in LPN loaded with melatonin at 10^{-9} M. In addition to that, it reduced apoptosis and ROS formation and increased cleavage rates. LPN applied in cell culture promotes the downregulation of pro-apoptotic factors such as CASP3, Bax, and SHC1 and upregulates antioxidative enzymes of SOD1, SOD2, GPX1, and MCL1.

Charão *et al.* (40) compared efficiency of melatonin-loaded LPN (0.93 mg/mL, EE of 32.11%) with free melatonin in alveolar epithelial A549 cell line for their antioxidant activity. They observed that melatonin-loaded LPN reduced cytotoxicity and genotoxicity to protect the cells against paraquat-induced oxidative stress better than free melatonin. Jin *et al.* (41) developed a synergistic nanoparticle with mesoporous polydopamine (mPDA) and tavilermide

(Tav) (mPDA-Mel-Tav) to load with melatonin. They used this nanoparticle to treat dry eye disease (DED) by scavenging ROS and promoting mucin production. The results showed that ROS level and apoptosis ratio were decreased by mPDA-Mel-Tav administration, indicating the central role of melatonin in ROS scavenging and antioxidant effect as well as the improvement effect of Tav on the production of mucins on the ocular surface.

Romeo *et al*. (42) also evaluated melatonin-loaded lipid-polymer hybrid as a protective agent in ocular diseases. They used PLGA-PEG polymers as the melatonin nanocarrier's core and coated it with a cationic lipid shell (MLT-LPHNs) and achieved positive surface charge (+39.8 mV) and high encapsulation efficiency (79.8 %). In an *in vitro* model of diabetic retinopathy, it was confirmed that this MLT-LPHNs significantly enhanced neuroprotective and antioxidant activities compared to melatonin aqueous solution at the same concentration. They also performed a Draize test to assess the tolerability and there was no sign of irritation. Ishaniya *et al*. (43) used polymer-supported liposome to load melatonin has encapsulated into a polymer/lipid hybrid nanocarrier by the self-assembly of 10,12-pentacosadiynoic acid (PCDA) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) through thin-film hydration method. The conjugated yne-ene chain of the polymeric backbone of polydiacetylene (PDA) facilitates the formation of stable nanoaggregation. This nanocarrier showed alkaline phosphatase (ALP) activity and promoted the calcium deposition in mouse mesenchymal stem cells more efficient than free melatonin. It also s upregulated the expression of Runx2, type1 col mRNAs, and secretion of extracellular matrix proteins that are related to osteoblast differentiation. In another study, melatonin was loaded into NLC and chitosan-based microspheres. Melatonin-loaded NLCs were prepared by a hot homogenization technique, while NLC-loaded microspheres were produced by a spray-drying method and a hybrid system was produced. This hybrid prolonged melatonin release, had good flowability, and appropriate extent of fluid uptake. When this hybrid system was used to simulate wound fluid, it swelled and created a hydrogel layer. The lipid part attenuated the evaporative water loss indicating the potential to provide optimal hydration for moderate exuding wounds. The hybrid system was reported to be biocompatible with skin keratinocytes and fibroblasts and showed antimicrobial activity against *Staphylococcus aureus* (44). The biological effects of hybrid nanocarriers are summarized in Table 2.

Table 2. The biological effects of melatonin in different hybrid nanocarriers.

Hybrid Carriers	**Biological effects**
Melatonin loaded LPN in cell culture	Highest hatching rate, reduced apoptosis and ROS formation, down regulation of CASP3, Bax and SHC1, upregulation of SOD1, SOD2, GPX1, MCL1 (38)
Melatonin loaded LPN on epithelial A54 cell line	Less cytotoxicity and genotoxicity to protect paraquat- induced oxidative stress (39)
mPDA-Mel-Tav	Reduced ROS levels and apoptosis and enhanced mucins on the ocular surface (40)
PLGA-PEG polymers coated with Cationic lipid shell	Enhanced neuroprotective and antioxidant activities (41)
Polymer/lipid hybrid (PCDA, PDA, and DMPC)	Increase in ALP and Calcium deposition and elevated RUNX2 (42)

LPN: lipid polymeric nanocarrier, mPDA-Mel-Tav: mesoporous polydopamine-melatonin-tavilermide, PLGA-PEG: polylactic-co-glycolic acid-polyethelene glycol, PCDA: 10,12-pentacosadiynoic acid, PDA: polydiacetylene, DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphocholine.

4. NON- IONIC SURFACTANT-BASED VESICLES (NIOSOMES)

In this section, the nanostructures formed by non-ionic surfactant membranes will be discussed. Niosomes are unilayer or multilayer nanocarriers that can be either used to deliver hydrophilic or hydrophobic drugs. They have a wide spectrum of applications including dermal usage or targeted delivery. Niosomes are very stable but leakier than liposomes and upon freezing the size decreases and the permeability for KCL increases (5). Niosomes can be prepared with simple methods, require less production cost, and are stable over an extended period, thus it overcomes the major drawbacks of liposomes (45).

Temprom *et al.* (46) have encapsulated melatonin into niosome by preparing the niosome using a mixture of Span 60 and cholesterol in various molar ratios (2:1, 1:1, and 1:2). They identified a nanoparticle with high stability and suitable for drug release (especially the niosomes with 1:1 ratio). The EE of melatonin in niosomes ranged from 85.09% to 86.69% and the released profile of melatonin-loaded niosomes were prolonged, with a maximum release of 21% after 48 h.

In a study of rats (47), melatonin incorporated niosome was used to protect skin damage induced by ultraviolet light compared to the sunscreen product. Niosome-incorporated melatonin was produced by tween80/span80 mixture with the EE=58.42%. Analysis of rat skin showed 58% permeation of melatonin versus 7.4% permeation of octyl methoxy cinnamate. and after 24 hours of ultraviolet light exposure, 37% of melatonin was still accumulated in the skin and showed high antioxidant activity. According to Priprem *et al*. (48), niosome gel containing melatonin can improve the pharmacokinetics of exogenous melatonin. This gel exhibited maximum adhesiveness for more than 3 hours at 37°C and had higher absorption and prolonged systemic circulation of melatonin. Another study compared the anti-candidiasis and anti-inflammatory effects of glutaryl melatonin and melatonin-loaded niosomes against oral mucositis induced by 5-fluorouracil (5-FU) in mice (49). The results showed that glutaryl melatonin incorporated in niosomes was more efficiency than that of glutaryl melatonin alone. Uthaiwat *et al*. (50) investigated the efficacy of melatonin niosome gel (MNG) and succinyl melatonin niosome gel (SNG) in small intestinal mucositis induced by 5-FU in mice. They observed decreased body weight and food consumption in all 5-FU-injected groups compared with the normal group. The MNG and SNG treatments maintained the food consumption and the normal integrity of the small intestines. They also observed that SNG reduced IL-1β to a level that was not different from the level in the normal groups.

5. SILICA-BASED NANOCARRIERS

Inorganic substances have significant use in drug delivery systems. Silica is one of these nanomaterials that can be applied in drug delivery for the treatment of pathological conditions. Silica has several advantages serving as a drug delivery system including its low toxicity, biocompatibility, unique optical properties, good adsorption and encapsulation features. These characteristics are the reason to use it in different medical situations, biosensor development, and cellular uptake (5). Silica has high surface-area-to-volume ratio to promote the molecules to agglomerate and functionalizing the surface with physical or chemical interaction in solution (51). For example, the silica-based nanocarrier was functionalized with diamine polymer, carboxy methyl B-cyclodextrin, and folic acid and was used as the thymoquinone-melatonin (TQ-MLT) delivery system. Five suspensions of silica nanoparticles at dose of 0.05 mg/ml were incubated with HeLa cells overnight. The results showed that as the polymer length decreased, TQ-MLT release was increased and the toxicity for HeLa cells increased with the length of polymer (52). Chen and colleagues (53) used mesoporous silica nanoparticles (MSN) and MSN-chitosan (MSN-CS) as carriers to deliver melatonin (MT) into rice plants.

They observed a controlled release for both nanocarriers but, MSN-CS performed better. They reported that these nanocarriers can reduce cadmium (Cd) concentration in rice leaves by 43.8%. It improved leaf photosynthesis efficiency, decreased malondialdehyde (MDA) content, enhanced the activity of antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) and also regulated the expression of Cd transport genes such as OsNramp1, OsNramp5, OsHMA2, OsHMA3, OsLCD, and OsPCR1.

6. GRAPHENE-BASED NANOMATERIALS

Graphene is a nanomaterial that is derived from carbon and can be used in drug delivery systems. Graphene oxide (GO) and reduced graphene oxide (rGO) are popular derivatives in studies that use graphene to produce nanocarriers (5). Because of hydrophilicity, solubility, and stability in colloids, graphene oxide has the ability to be used in drug delivery systems and be functionalized with several other molecules. The differences in these nanomaterials are the layer numbers of GO and rGO and the molecules of their surfaces (54).

To evaluate the effects of melatonin and doxorubicin co-delivery on osteosarcoma cells, a functionalized graphene-dendrimeric system was designed via Fe_3O_4 nanoparticle (NP) as a magnetic nanocarrier. β-Cyclodextrin (β-CD) was modified by creating amine functional groups and then grafted with graphene oxide (GO) (EE for DOX=99.92% and EE for melatonin=21.5%). The human osteosarcoma cell lines including Saos-2 and MG-63, as well as human bone marrow mesenchymal stem cells (hBM-MSC) were used to test the effectiveness of this system. The results showed the good biocompatibility and effective anticancer performance. This graphene-dendrimeric system caused down-regulation of X-linked Inhibitor of Apoptosis (XIAP), survivin, and human telomerase catalytic subunit (hTERT) (55).

7. CHITOSAN-BASED NANOPARTICLES

Chitosan is a linear semi-synthetic amino polysaccharide that is primarily made by deacetylation of chitin. Its carbon backbone is similar to cellulose backbone except it has an amino group on C2. Chitosan is soluble in acidic environments and not soluble in alkaline or neutral pH (56). One of the most common formulation of chitosan is produced by an interaction between aminosugar monomers and polyanions(57). Chitosan is non-immunogenic, biocompatible, and enzymatic biodegradable. It can also have anti-cancer and cholesterol-lowering and wound healing effects (56). One reason for using melatonin-loaded nanoparticles is to enhance the distribution of melatonin in cells and to increase its stability during exposure to air and light. Study has evaluated the long-term stability of melatonin-loaded chitosan/lecithin nanoparticles in different terms of lyophilization. The best results were achieved when lyophilization was done with trehalose 2.5% w/v (7 months stable at 4°C) (58). To evaluate the toxicity of chitosan, Hafner and his colleagues (59) used chitosan/lecithin nanoparticles at dose of 400 µg/ml on Caco-2 and U87MG cells. NPs didn't have any negative effect on the cell plasma membrane and cell survival. In additional study by the same group.(60), HaCat cell line and BJ fibroblast cells were used to evaluate the potential toxicity of chitosan. The results showed that even at the dose of 200 µg/ml, chitosan/lecithin nanocarriers was without any toxicity to the tested cells. Concentration-dependent toxicity of chitosan is usually seen in free and soluble forms of this semi-synthetic polysaccharide and when it is structured as nanocarrier its cytotoxicity is significantly decreased. The most common material used in producing chitosan-based nanocarriers for melatonin delivery is lecithin due to the improvement of adhesiveness, penetration and melatonin release (5).

Shokrzade and colleagues (61) examined the effect of melatonin-loaded nanoparticles which were based on chitosan and tripolyphosphate on HepG2 cell line. They used etoposide to induce genotoxicity in these cells. They observed that melatonin-loaded nanocarriers reduced the harmful effects of etoposide by reducing DNA damage and oxidation.

Jafari *et al*. (62) produced a pH-sensitive nanoparticle consisting of chitosan (CTS) and hydroxypropyl methylcellulose (HPMC) and loaded it with melatonin and used it on MDA-MB-231 breast cancer cells. They reported that melatonin-loaded CTS/HPMC NPs had higher cytotoxicity against MDA-MB-231 cancer cells than the free MLT.

To evaluate melatonin's anti-inflammatory action in Inflammatory Bowel Disease (IBD), Soni *et al*. (63) prepared melatonin-loaded chitosan nanoparticles (Mel-CSNPs). These nanoparticles showed a better anti-inflammatory effect than free melatonin by reducing nitric oxide, inhibiting nuclear translocation of NF-kB p65, and reducing IL-1β and IL-6 expression. In an *in vivo* model, Mel-CSNPs caused a reduction in disease activity parameters and inhibited neutrophilic infiltration. Histochemical analysis showed a reduction of inflammatory markers such as nitric oxide synthase-2 and nitro tyrosine (64). In a study, a melatonin loaded lecithin-chitosan nanoparticle was formulated by adding melatonin and an ethanolic solution containing soybean lecithin to an aqueous solution that contained chitosan, then, went under sonication. The process generated the nanoparticles with a size of 160 nm and a zeta potential of 25 mV and an entrapment efficiency of 27%. This Mel-NP was administered to diabetic rats and the results showed the improved wound healing capacity of Mel-NP in these animals indicated by wound closure earlier than other treatments, induction of fibroblast and angiogenic proliferation accompanied by expressive collagen deposition.

Aghaz *et al*. (65) prepared an amphiphilic chitosan nanocarrier loaded with melatonin and tretinoin by self-assembled method to evaluate the effect of their synergistic antioxidant effect in mice oocytes/embryos. The results of their study demonstrated that the dual delivery of Mel and TTN could accumulate a safe (without high-dose toxicity) synergistic anti-oxidative effect in oocyte/embryo and inhibited intra/extracellular ROS levels by an enhanced intracellular penetration. The beneficial effects of melatonin-loaded chitosan-based nanoparticles were summarized in Table 3,

Table 3. The results of using melatonin-loaded chitosan-based nanoparticles in different conditions.

Conditions of Chitosan Based Nanoparticles Used	**Biological effects**
HepG2 Cell line	Reduced toxicity induced by etoposide indicated by reducing DNA damage and oxidation (60)
Inflammatory Bowel Disease	Reduced nitric oxide, IL-1β and IL-6 and nitrotyrosine. Inhibited nuclear translocation of NF-kB p65 (62)
Diabetic Rat models	Wound healing features such as earlier closure of wound and improvement of fibroblastic proliferation (63)
mice oocytes/embryos	Co-delivery with tretinoin resulted in more effective antioxidant actions and inhibit ROS levels (64)

8. SYNTHETIC POLYMERIC NANOPARTICLES

Different kind of polymers are used to produce nanoparticles for melatonin delivery (hydrophobic or hydrophilic). They are popular for targeted drug delivery and they can increase

the half-life of the substances besides reducing the side effects. They can be incorporated with drugs chemically bonding to them. Each synthetic polymer can make the drug delivery specific because of the different properties in the physical or chemical features of nanoparticles including size, shape, surface charge, or encapsulation efficiency. A variety of synthetic polymers have been used for melatonin delivery. These include poly-caprolactone, poly-lactic acid, poly-lactic co- glycolic acid, polyethylene glycol, and poly-methacrylic acid-co-methyl methacrylate and all of them are capable of loading melatonin.

9. POLY-CAPROLACTONE AND MELATONIN

The first synthetic polymer discussed in this review is poly-caprolactone (PCL). Different methods such as nanoprecipitation, electrospraying, interfacial deposition, and electrospinning can make PCL nanoparticles.

Melatonin was encapsulated in PCL nanoparticles by electrospraying technique and the 3 wt. % solution of melatonin-PCL nanocarrier with EE=73% had a prolonged release of melatonin for about 8 hours. When this preparation was incubated with human osteoblast cells or administered to female Sprague Dawley rats it significantly increased the population of cells or increased the bone volume of rats compared to the control (66).

The melatonin-loaded PCL nanocarriers was used delivery of melatonin from nose-to-brain for the treatment of glioblastoma. The results showed that administration of this nanocarriers intranasally could rapidly translocated to the brains of rats and the enhanced cytotoxic effects against U87MG cells were achieved indicating these nanoparticles being suitable for the treatment of glioblastoma (67).

Another method to prepare PCL nanoparticles is electrospinning. Song and colleagues (68) used this method to produce melatonin-loaded aligned PCL with electrospun fibrous membrane. These NPs were reconstructed to the structure of tendon to bone insertion site. The results showed the enhancement of chondrocytes differentiation to chondroid pellet. And after implanting these membranes in rats they achieved inhibition of macrophage infiltration, promotion of collagen maturation and reduction of fibrovascular tissue, as well as promotion injuried tissue recovery.

Zhang and colleagues (69) produced a The melatonin-loaded magnesium-polycaprolactone (Mg-PCL) was also used to evaluate its role in the cell in cell (CIC) structures in osteosarcoma (OS). This preparation significantly inhibited the key CIC pathway, Rho/ROCK, through the cAMP/PKA signaling pathway, interfering with the mitochondrial physiology of OS cells, and thus playing an anti-invasion and anti-metastasis role in OS. Therefore, this melatonin-loaded Mg–PCL scaffolds had the capacity inhibited the proliferation, invasion, and metastasis of OS cells through the CIC pathway.

10. POLY-LACTIC AND POLY-LACTIC-CO-GLYCOLIC ACID AND MELATONIN

Evidence showed that HPLC with photodiode array is safe and suitable method to determine melatonin encapsulation efficiency in poly-lactic acid (PLA) nanocarriers (70). The PLA-melatonin NPs were prepared by Pandey *et al.* (71) with emulsification /nanoprecipitation method with EE=78% being analyzed with HPLC the effects of melatonin-loaded PLA nanoparticles versus melatonin on ROS formation and blastogenic responses were tested in splenocytes. The results indicated that melatonin-loaded PLA NPs had better effects on the improvement of immune response by increasing blastogenic response and reducing ROS formation that of melatonin alone.

Poly-lactic-co-glycolic acid (PLGA) is a co polymer of lactic acid and glycolic acid which has many properties including biocompatibility, the ability to deliver both hydrophilic and

hydrophobic drugs, protecting the drug from biodegradation, prolonging the half-life of the NP, and the ability to be modified by other molecules (72). By use of emulsion-diffusion-evaporation method, Altındal *et al*. (73) formulated melatonin-loaded PLGA (0.2% w/v) and micro particles and used them to treat osteosarcoma MG-63 cell line to induce cytotoxicity. This melatonin-loaded microparticles (carrying 1.7 µg melatonin) had more inhibitory effects on this tumor cell line than that of melatonin alone. Jarrar *et al*. (74) prepared a melatonin-loaded PLGA by double emulsion solvent evaporation method and then loaded into chitosan/hydroxyapatite (HAp) scaffolds and then, they examined the effect of BMP-2 and this nanocarrier on pre-osteoblastic MC3T3-E1 cells. They observed that when PLGA microparticles (melatonin 10 µg/scaffold) and BMP-2 (20 ng/scaffold) are administered simultaneously, it can cause a higher number of cells and bone mineralization and high expression of Runt-related transcription factor 2 (RUNX2) and alkaline phosphatase (ALP).
PLGA is also a promising carrier to improve antioxidant activity of melatonin. Martins *et al*. (75) evaluated the antioxidant action of melatonin in erythrocytes by use of melatonin-loaded PLGA produced by emulsion solvent evaporation method. It was shown that melatonin-loaded PLGA showed no hemolysis reaction and had better scavenging activity in comparison to free melatonin.

Pan *et al*. (76) used a co-delivery system of dexamethasone (Dex) and melatonin-loaded PLGA to treat glaucoma. They produced these nanoparticles by co-axial electrospray method (EE= more than 85%) and used it on R28 cells. The showed that the nanoparticles enhanced the penetration, reduced the intraocular pressure, and had no toxicity on R28 cells. It could be concluded that DEX-ML-PLGA had profound effect for management of glaucoma. In a study to treat osteosarcoma, melatonin-loaded PLGA was incorporated into chitosan/hydroxyapatite (chitosan/HAp) scaffolds. This system showed a biphasic melatonin release-pattern with a burst release within 24 h, and then a sustained release for 40 days which caused an increase in the expressions of osteogenic differentiation markers in preosteoblastic MC3T3-E1 cells. Also, melatonin/2-hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complex was prepared by microwave technology to inhibit MG-63 osteosarcoma cells. This complex was incorporated into the chitosan/HAp scaffolds and loaded melatonin was rapidly released within 5 h and inhibited the proliferation of MG-63 cells in the G_0/G_1 phase (77).

11. POLYETHYLENE GLYCOL AND MELATONIN

Chen *et al*. (78) used oil in water emulsion method to generate melatonin-loaded polyethylene glycol (PEG) nanocarriers and polypropylene sulfide, then these nanocarriers were used to treat sepsis-induced liver injury. These NPs showed higher anti-inflammatory effects due to their suitable biocompatibility and higher antioxidant capacity indicated by reduced ROS formation and less damage tissue in treated animals compared to the control. Li Volti *et al*. (79) also examined the effect of melatonin-loaded PLGA (NP 1) and melatonin-loaded PLGA-PEG (NP 2) on sepsis animal model. The oxidative stress was accessed by the expression of heme oxygenase-1 (HO-1), lipid hydroperoxide (LOOH) level, and total thiol groups in tissue homogenates of animals. The results showed that both NP1 and NP2 upregulated the thiol groups and reduced LOOH levels. In addition, NP2 caused a great reduction of HO-1 expression and had better antioxidant activity than NP1.
The PLGA-monomethoxy-poly-PEG (PLGA-mPEG)-melatonin NP with EE=79.3% was used to evaluate the protective effect on adipose-derived mesenchymal stem cells (ADSCs) (). This NP reduced formation of p53-cyclophilin D complex and rescued the ADSCs from hypoxia/reoxygenation injury. Also, administration of melatonin-loaded PLGA-mPEG showed a higher ADSC survival rate than melatonin alone after myocardial infarction (80).

12. POLY METHACRYLIC ACID-CO-METHYL METHACRYLATE AND MELATONIN

Poly methacrylic acid-co-methyl methacrylate or poly MMA-co-MAA is a biocompatible polymer that does not produce toxic residues when it is solved in water. Schaffazick *et al*. (81) loaded melatonin (1 or 10 mg/kg) into poly MMA-co-MAA and coated it with polysorbate 80 then administered it to mice intraperitoneally. It was reported that this NP reduced lipid peroxidation in mice's brain and liver and also improved the total antioxidant capacity.

13. CONCLUSION

Nanocarriers can be used to deliver active ingredients to more effectively treat disorders with reduced side effects. Specifically, to melatonin, many studies have demonstrated that liposomal carriers can improve the penetration of melatonin to targeted tissue and also can enhance the antioxidant effects of melatonin. SLN and NLC can be administered in different routes such as locally, orally or ocularly and they also can improve the anti-apoptotic and antioxidant effects of melatonin. The field of hybrid carriers for melatonin delivery has the advantage of enhanced loading capacity and improved permeation, therefore, they can be used to deliver melatonin to enhance its neuroprotective, wound healing and bone regeneration activities. Other types of nanocarriers can also be selected to deliver melatonin depending on the treatment purpose, tissue specific and properties of the study. It is well established that incorporating melatonin into these nanocarriers leads to improved performance of melatonin as an antioxidant, anti-inflammatory or anti-tumor agent. These melatonin-loaded nanocarriers can be used as an adjuvant or the primary active molecule to prevent and treat many diseases with significantly increased efficiency. Although the complete spectrum of functions of melatonin-loaded nanoparticles is currently unknown, some of them have been chemically optimized for use. However, aspects such as optimal dosage, timing, administration route, responses of other cells or the specific effects of melatonin in nanocarriers in various diseases should be evaluated continuously in the future.

ACKNOWLEDGMENTS

The author is grateful to Golestan University of Medical Sciences, Gorgan, Iran, for providing all kinds of facilities to prepare this manuscript.

AUTHORSHIP

SMM designed the idea for the article. AAJ, FM and SA performed the literature search and wrote the first draft of the manuscript. ZAB reviewed and revised the manuscript. SMM reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

REFERENCES

1. Laurent S, *et al*. (2010) Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chem. Rev.* **110** (4): 2574-2574.

2. Jeevanandam J, *et al.* (2018) Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein J. Nanotechnol.* **9**: 1050-1074.
3. Stamatoiu O, *et al.* (2012) Nanoparticles in liquid crystals and liquid crystalline nanoparticles. *Top. Curr. Chem.* **318**: 331-393.
4. Rakotoarisoa M, *et al.* (2019) Cubic liquid crystalline nanostructures involving catalase and curcumin: BioSAXS study and catalase peroxidatic function after cubosomal nanoparticle treatment of differentiated SH-SY5Y cells. *Molecules* **24** (17): 3058.
5. Chuffa LGA, *et al.* (2021) Melatonin-loaded nanocarriers: New horizons for therapeutic applications. *Molecules* **26** (12):3562.
6. Mir SM, *et al*. (2022) Melatonin: A smart molecule in the DNA repair system. *Cell. Biochem. Funct.* **40** (1): 4-16.
7. Mirza Aghazadeh Attari M, *et al.* (2020) Melatonin: an important anticancer agent in colorectal cancer. *J. cell. physiol.* **235** (2): 804-817.
8. Heydari N, *et al*. (2023) Melatonin: An anticancer molecule in esophageal squamous cell carcinoma: A mechanistic review. *Melatonin Res.* **6** (1): 59-71.
9. Targhazeh N, *et al*. (2022) Oncostatic activities of melatonin: Roles in cell cycle, apoptosis, and autophagy. *Biochimie* **202**: 34-48.
10. Asanjani Oskoii M, *et al*. (2022) Serum level of melatonin in patients with osteoarthritis and its relation with 8-hydroxy-2-deoxyguanosine and vitamin D. *J. Res Clin. Med.* **8** (1): 34-34.
11. Wang S-Y, *et al* (2020) Indole-based melatonin analogues: Synthetic approaches and biological activity. *Eur. J. Med. Chem.* **185**: 111847.
12. Pandi-Perumal SR, *et al.* (2008) Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. *Prog. Neurobiol.* **85** (3): 335-53.
13. Mir SM., *et al.* (2020) The sensitization of melatonin in osteosarcoma cells by suppression of anti-apoptotic proteins. *Pharm. Sci.* **26** (2): 159-164.
14. Wade AG, *et al.* (2010) Nightly treatment of primary insomnia with prolonged release melatonin for 6 months: a randomized placebo controlled trial on age and endogenous melatonin as predictors of efficacy and safety. *BMC Med.* **8**: 51.
15. Zetner D, Rosenberg J (2020) Solubility and stability of melatonin in propylene glycol, glycofurol, and dimethyl sulfoxide. *F1000Res*. doi.10.12688/f1000research.21992.1. PPR: PPR11181.
16. Zhang Q, *et al.* (2017) Construction of nanoscale liposomes loaded with melatonin via supercritical fluid technology. *J. Microencapsul.* **34** (7): 687-698.
17. Molska A, *et al.* (2020) In vitro and in vivo evaluation of organic solvent-free injectable melatonin nanoformulations. *Eur. J. Pharm. Biopharm.* **152**: 248-256.
18. Lin W, *et al.* (2019) Poly-phosphocholinated liposomes form stable superlubrication vectors. *Langmuir*. **35** (18): 6048-6054.
19. Gonçalves MC, *et al*. (2012) Chitosan coated liposomes as an innovative nanocarrier for drugs. *J. biomed. nanotechnol.* **8** (2): 240-250.
20. Dubey V, *et al.* (2007) Melatonin loaded ethanolic liposomes: physicochemical characterization and enhanced transdermal delivery. *Eur. J. Pharm. Biopharm.* **67** (2): 398-405.
21. Marepally S, *et al.* (2013) Design, synthesis of novel lipids as chemical permeation enhancers and development of nanoparticle system for transdermal drug delivery. *PLoS One* **8** (12): e82581.
22. Sana S, *et al.* (2017) Vesicular melatonin efficiently downregulates sodium fluoride-induced rat hepato- and broncho-TNF-α, TGF-β expressions, and associated oxidative injury: a comparative study of liposomal and nanoencapsulated forms. *Int. J. Nanomedicine.* **12**: 4059-4071.

23. Bseiso EA, *et al.* (2022) Nose to brain delivery of melatonin lipidic nanocapsules as a promising post-ischemic neuroprotective therapeutic modality. *Drug Deliv.* **29** (1): 2469-2480.
24. Hou X, *et al.* (2022) Application and efficacy of melatonin elastic liposomes in photoaging mice. *Oxid. Med. Cell. Longev.* 2**022**: 7135125.
25. Alsaad AA, Hussien AA, Gareeb MM, (2020) Solid lipid nanoparticles (SLN) as a novel drug delivery system: A theoretical review. *Syst. Rev. Pharm.* **11**: 259-273.
26. López KL, *et al.* (2023) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) prepared by microwave and ultrasound-assisted synthesis: Promising green strategies for the nanoworld. *Pharmaceutics* **15** (5): 1333.
27. Albertini B, *et al*. (2014) Formulating SLMs as oral pulsatile system for potential delivery of melatonin to pediatric population. *Int. J. Pharm.* **469** (1): 67-79.
28. Musazzi UM, *et al.* (2019) A new melatonin oral delivery platform based on orodispersible films containing solid lipid microparticles. *Int. J. Pharm.* **559**: 280-288.
29. Tursilli R, *et al.* (2006) Enhancement of melatonin photostability by encapsulation in lipospheres. *J. Pharm. Biomed. Anal.* **40** (4): 910-914.
30. Hatem S, *et al.* (2018) Clinical cosmeceutical repurposing of melatonin in androgenic alopecia using nanostructured lipid carriers prepared with antioxidant oils. *Expert Opin. Drug Deliv.* **15** (10): 927-935.
31. Rezzani R, *et al.* (2009) Melatonin delivery in solid lipid nanoparticles: prevention of cyclosporine A induced cardiac damage. *J. Pineal Res.* **46** (3): 255-261.
32. Mirhoseini M, *et al*. (2019) Protective effects of melatonin solid lipid nanoparticles on testis histology after testicular trauma in rats. *Res. Pharm. Sci.*. **14** (3): 201-208.
33. Sabzichi M, *et al.* (2016) Sustained release of melatonin: A novel approach in elevating efficacy of tamoxifen in breast cancer treatment. *Colloids Surf. B. Biointerfaces* **145**: 64-71.
34. Parvez S, *et al.* (2023) Coalition of biological agent (melatonin) with chemotherapeutic agent (amphotericin B) for combating visceral leishmaniasis via oral administration of modified solid lipid nanoparticles. *ACS Biomater. Sci. Eng.* **9** (6): 2902-2910.
35. Aghaz F, *et al.* (2021) Enhanced cryoprotective effect of melatonin and resveratrol by coencapsulation: Improved in vitro development of vitrified-warmed mouse germinal vesicle oocytes. *Biopreserv. Biobank* **19** (3): 184-193.
36. Hallan SS, *et al.* (2016) Lipid polymer hybrid as emerging tool in nanocarriers for oral drug delivery. *Artif. Cell. Nanomed. Biotechnol.* **44** (1): 334-49.
37. Pohlmann AR, *et al.* (2010) Preparation of drug-loaded polymeric nanoparticles and evaluation of the antioxidant activity against lipid peroxidation. *Methods Mol. Biol.* **610**: 109-121.
38. Carbone C, *et al.* (2016) Innovative hybrid vs polymeric nanocapsules: The influence of the cationic lipid coating on the "4S". C*olloids Surf. B. Biointerfaces*. **141**: 450-457.
39. Komninou ER, *et al*. (2016) Effects of two types of melatonin-loaded nanocapsules with distinct supramolecular structures: Polymeric (NC) and lipid-core nanocapsules (LNC) on bovine embryo culture model. *PLoS One* **11** (6): e0157561.
40. Charão MF, *et al*. (2015) Protective effects of melatonin-loaded lipid-core nanocapsules on paraquat-induced cytotoxicity and genotoxicity in a pulmonary cell line. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **784**: 1-9.
41. Jin K, *et al*. (2022) Anti-oxidative and mucin-compensating dual-functional nano eye drops for synergistic treatment of dry eye disease. *Appl. Mater. Today* **27**: 101411.
42. Romeo A, *et al.* (2022) Melatonin loaded hybrid nanomedicine: DoE approach, optimization and in vitro study on diabetic retinopathy model. *Int. J. Pharm.* **627**: 122195.

43. Ishaniya W, *et al*. (2022) Nano-encapsulation of melatonin into polydiacetylene-phospholipid assembly for sustained-release and enhanced bone formation in zebrafish. *J. Drug Deliv. Sci. Technol.* **72**: 103415.
44. Duvnjak Romić M, *et al*. (2019) Melatonin loaded lipid enriched chitosan microspheres – Hybrid dressing for moderate exuding wounds. *J. Drug Deliv. Sci. Technol.* **52**: 431-439.
45. Chen S, *et al.* (2019) Recent advances in non-ionic surfactant vesicles (niosomes): Fabrication, characterization, pharmaceutical and cosmetic applications. *Eur. J. Pharm. Biopharm.* **144**: 18-39.
46. Temprom L, *et al*. (2022) A novel preparation and characterization of melatonin loaded niosomes based on using a ball milling method. *Mater. Today Commun.* **31**: 103340.
47. Arslan Azizoglu G, *et al*. (2017) Dual-prevention for UV-induced skin damage: incorporation of melatonin-loaded elastic niosomes into octyl methoxycinnamate pickering emulsions. *AAPS Pharm. Sci. Tech*. **18** (8): 2987-2998.
48. Priprem A, *et al*. (2018) Transmucosal delivery of melatonin-encapsulated niosomes in a mucoadhesive gel. *Ther. Deliv*. **9** (5): 343-357.
49. Damrongrungruang T, *et al*. (2020) Glutaryl melatonin niosome gel for topical oral mucositis: Anti- inflammatory and anticandidiasis. *Curr. Drug Deliv*. **17** (3): 195-206.
50. Uthaiwat P, *et al*. (2021) Oral administration of melatonin or succinyl melatonin niosome gel benefits 5-FU-induced small intestinal mucositis treatment in mice. *AAPS Pharm. Sci. Tech.* **22** (5): 200.
51. Bitar A, *et al*. (2012) Silica-based nanoparticles for biomedical applications. *Drug Discov. Today* **17** (19-20): 1147-1154.
52. Khattabi AM, Talib WH, Alqdeimat DA, (2018) The effect of polymer length on the in vitro characteristics of a drug loaded and targeted silica nanoparticles. *Saudi Pharm. J.* **26** (7): 1022-1026.
53. Chen J, *et al.* (2022) Development of melatonin nano-delivery systems to reduce cadmium accumulation in rice (Oryza sativa L.) seedlings: Insights from photosynthetic efficiency, antioxidative response and gene expression. *Environ. Exp. Bot*. **196**: 104822.
54. Usman MS, *et al*. (2018) Graphene oxide as a nanocarrier for a theranostics delivery system of protocatechuic acid and gadolinium/gold nanoparticles. *Molecules* 23 (2): 500.
55. Niu G, *et al*. (2021) Melatonin and doxorubicin co-delivered via a functionalized graphene-dendrimeric system enhances apoptosis of osteosarcoma cells. *Mater. Sci. Eng*. **119**: 111554.
56. Rahimi M, *et al*. (2022) Chitosan-based biomaterials for the treatment of bone disorders. *Int. J. Biol. Macromol.* **215**: 346-367.
57. Saeed RM, Dmour I, Taha MO, (2020) Stable chitosan-based nanoparticles using polyphosphoric acid or hexametaphosphate for tandem ionotropic/covalent crosslinking and subsequent investigation as novel vehicles for drug delivery. *Front. Bioeng. Biotechnol*. **8**: 4.
58. Hafner A, *et al*. (2011) Short- and long-term stability of lyophilised melatonin-loaded lecithin/chitosan nanoparticles. *Chem. Pharm. Bull. (Tokyo)* **59** (9): 1117-1123.
59. Hafner A, *et al*. (2009) Melatonin-loaded lecithin/chitosan nanoparticles: physicochemical characterisation and permeability through Caco-2 cell monolayers. *Int. J. Pharm.* **381** (2): 205-213.
60. Hafner A, *et al.* Lecithin/chitosan nanoparticles for transdermal delivery of melatonin. *J. Microencapsul.* 28 (8): 807-815.
61. Shokrzadeh M, Ghassemi-Barghi N (2018) Melatonin loading chitosan-tripolyphosphate nanoparticles: Application in attenuating etoposide-induced genotoxicity in HepG2 cells. *Pharmacol.* **102** (1-2): 74-80.

62. Jafari H, *et al*. (2021) Characterization of pH-sensitive chitosan/hydroxypropyl methylcellulose composite nanoparticles for delivery of melatonin in cancer therapy. *Mater. Lett.* **282**: 128818.
63. Soni JM, *et al*. (2021) Melatonin-loaded chitosan nanoparticles endows nitric oxide synthase 2 mediated anti-inflammatory activity in inflammatory bowel disease model. *Mater. Sci. Eng. C. Matter. Biol. Appl*. **124**: 112038.
64. Lopes Rocha Correa V, *et al*. (2020) Melatonin loaded lecithin-chitosan nanoparticles improved the wound healing in diabetic rats. *Int. J. Biol. Macromol*. **162**: 1465-1475.
65. Aghaz F, *et al*. (2021) Enhanced synergistic-antioxidant activity of melatonin and tretinoin by co-encapsulation into amphiphilic chitosan nanocarriers: During mice in vitro matured oocyte/morula-compact stage embryo culture model. *Reprod. Sci*. **28** (12): 3361-3379.
66. Gurler EB, *et al*. (2019) Encapsulated melatonin in polycaprolactone (PCL) microparticles as a promising graft material. *Mater. Sci. Eng. C. Mater. Biol. Appl.* **100**: 798-808.
67. de Oliveira Junior ER, *et al*. (2019) Increased nose-to-brain delivery of melatonin mediated by polycaprolactone nanoparticles for the treatment of glioblastoma. *Pharm. Res.* 36 (9): 131.
68. Song W, *et al*. (2019) Pro-chondrogenic and immunomodulatory melatonin-loaded electrospun membranes for tendon-to-bone healing. *J. Mater. Chem. B.* **7** (42): 6564-6575.
69. Zhang W, *et al*. (2021) 3D-printing magnesium–polycaprolactone loaded with melatonin inhibits the development of osteosarcoma by regulating cell-in-cell structures. *J. Nanobiotechnol.* **19** (1): 263.
70. Martins LG, Khalil NM, Mainardes RM (2017) Application of a validated HPLC-PDA method for the determination of melatonin content and its release from poly(lactic acid) nanoparticles. *J. Pharm. Anal.* **7** (6): 388-393.
71. Pandey SK, *et al*. (2015) Synthesis and in vitro evaluation of melatonin entrapped PLA nanoparticles: an oxidative stress and T-cell response using golden hamster. *J. Biomed. Mater. Res. A.* **103** (9): 3034-44.
72. Danhier F, *et al*. (2012) PLGA-based nanoparticles: An overview of biomedical applications. *J. Control Release.* **161** (2): 505-522.
73. Altındal D, Gümüşderelioğlu M (2016) Melatonin releasing PLGA micro/nanoparticles and their effect on osteosarcoma cells. *J. Microencapsul.* **33** (1): 53-63.
74. Jarrar H, Çetin Altındal D, Gümüşderelioğlu M, (2021) Scaffold-based osteogenic dual delivery system with melatonin and BMP-2 releasing PLGA microparticles. *Int. J. Pharm.* **600**: 120489.
75. Martins LG, Khalil NM, Mainardes RM, (2018) PLGA nanoparticles and polysorbate-80-coated PLGA nanoparticles increase the in vitro antioxidant activity of melatonin. *Curr. Drug Deliv.* **15** (4): 554-563.
76. Pan X, *et al*. (2020) Co-delivery of dexamethasone and melatonin by drugs laden PLGA nanoparticles for the treatment of glaucoma. *J. Drug Deliv. Sci. Technol.* **60**: 102086.
77. Çetin Altındal D, Gümüşderelioğlu M, (2019) Dual-functional melatonin releasing device loaded with PLGA microparticles and cyclodextrin inclusion complex for osteosarcoma therapy. *J. Drug Deliv. Sci. Technol.* **52**: 586-596.
78. Chen G, *et al.* (2017) Reactive oxygen species-responsive polymeric nanoparticles for alleviating sepsis-induced acute liver injury in mice. *Biomaterials* **144**: 30-41.
79. Li Volti G, *et al*. (2012) Antioxidant potential of different melatonin-loaded nanomedicines in an experimental model of sepsis. *Exp. Biol. Med. (Maywood)* **237** (6): 670-677.
80. Ma Q, *et al.* (2018) Poly(Lactide-Co-Glycolide)-monomethoxy-poly-(polyethylene glycol) nanoparticles loaded with melatonin protect adipose-derived stem cells transplanted in infarcted heart tissue. *Stem Cells* **36** (4): 540-550.

81. Schaffazick SR, *et al*. (2008) Incorporation in polymeric nanocapsules improves the antioxidant effect of melatonin against lipid peroxidation in mice brain and liver. *Eur. J. Pharm. Biopharm.* **69** (1): 64-71.

Please cite this paper as:
Ahmadi Jazi, A., Mohammadzadeh, F., Amirkhanlou, S., Arab Bafarani, Z. and Mir, S.M. 2023. Nanocarriers for melatonin delivery. Melatonin Research. 6, 4 (Dec. 2023), 503-519. DOI:https://doi.org/https://doi.org/10.32794/mr112500165.