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Epi-drug-Loaded Niosomes in Combination with Blepharis Persica Extract Improves Spinal Cord Injury in Male Rat

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A B S T R A C T

1) Background: Taxol facilitates nerve regeneration but it has unwanted side-effects via the tumor suppressive function of miR-34a which induces XIST expression and affects paclitaxel administration. We aimed to examine the neuroprotective effect of Paclitaxel-loaded niosomes (PLNs) to enhance fertility by combining with the *Belpharis Persica* (B.P) extract in male Wistar rat model of spinal cord injury (SCI). 2) Methods: PLNs were prepared using the thin-film hydration method. After laminectomy at thoracic level (T9/T10) SCI was induced using the weight drop method. Rat treated with either PLNs (2.5mg/kg), PLNs (2.5mg/kg)+B.P extract (300mg/kg-14 days), B.P extract (300mg/kg-14 days), Normal, SCI (normal saline), and sham (laminectomy). 3) Results: The transmission electron microscopy TEM and laser diffraction methods were confirmed the existence of spherical vesicles with feature sizes about 200 nm. The various behavioral, pathological and molecular parameters (CATSPER gene expression) were distinguished. SCI resulted in a significant decrease ($P<0.05$) in sperm motility, behavioral skills, and CATSPER genes expression were all regenerated by treatment with combination therapy (PLNs+B.P extract) in comparison to the others. Besides, the increased in sperm motility, and CATSPER genes expression were significantly ($P<0.05$) demonstrated in PLNs and B.P treated groups. 4) Conclusion: Taken together, the results indicated that the PLNs and B.P groups showed a significant increase in sperm parameters, but combination therapy caused the greater recovery of both gene expression and motor functions to decrease the neurological deficits no serious adverse reactions and enhanced fertility.

Introduction

Spinal Cord Injury (SCI) is a trauma, along with numerous consequences. The most important causes of trauma to the spinal cord are accidents, the bullet hit, tumor, maternity disorders of spinal cord degenerative diseases, and surgery. Approximately 62% of SCI's are youths aged 15-30 which males are about 80% of the patient's group.

SCI can affect the male reproductive system. Various investigations studied the effects of the SCI on semen parameters (Sinha, 2017). In this regard, scientists examined the rate of spermatogenesis both short (2 weeks) and long-term (20 weeks) after SCI in adult rats. They found that SCI may lead to problems in sperm

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production even in the short-time after SCI (Anderson, 2018). Huang et al. (1998) reported that changes in spermatogenesis in adult rats with SCI occurred in the long-term (after four weeks) (Huang, 1998). The serum level of estrogen in rat model of SCI accelerates within a month thus the levels of spermatogenesis declines. Although hormone therapy facilitates the process of spermatogenesis, but sometimes spermatogenesis was arrested in adult rats after SCI. These results determined that SCI is responsible for the returnable procedure of spermatogenesis (Whashburn et al, 2021). On the other hand, Sperm motility is related to the activation of signaling pathways that affect movement. These pathways are mainly linked to calcium ion. Besides, *CATSPER 1-4* gene family encodes unique calcium channels in sperm (Nowicka-Bauer et al, 2021). *CATSPER1* gene is essential for male rat fertility, sperm motility, and penetration of sperm into the Ovum. The *CATSPER2* gene also plays a key role in spermatogenesis and sperm motility. Deletions of this region remove *CATSPER2*-related male infertility gene in animals (Sun et al, 2017).

Scientists showed that the mature state of chromatin in different cells of the central nervous system (CNS) may affect the CNS pathophysiology, and silenced developmental gene expression can change histone acetylation could and can be used in novel approaches to stimulate endogenous repair of CNS. Epigenetic modifications affect many fundamental cellular processes are affected such as some essential aspects of the neuronal regeneration (Penas et al, 2018). Recently, paclitaxel has been used to treat SCI. It facilitates the aggregation of microtubules by stabilizing the tubulin dimers and preventing depolymerization of them (Yin, 2018). But paclitaxel can cause unwanted effects in some patients. The drug is metabolized with a short half-life, so repeated use of relatively high doses is necessary in order to achieve the required concentration over appropriate time periods, which increases risks for toxicity and covalent modification of histones as one of the most important of the primary epigenetic mechanisms (Penas et al, 2018, Yin, 2018). The use of carriers to improve efficiency and the reduction of paclitaxel toxicity seems promising. One of the most controversial issues with the use of niosomes and liposomes is the complete reduction or elimination of drug side effects (Sabry et al, 2021).

Besides, the use of medicinal herbs has a long history due to the lower side effects, simpler access, easier acceptance and cost-effectiveness. Recently, the use of herbal medicines simultaneously or alone has been considered to reduce side effects of chemical drugs (Cao, 2018). Due to the valuable properties of native Iranian medicinal herbs, *Blepharis persica* (*B.P*) was chosen. B.P is a traditional Iranian medicinal herb which has been used to enhance male fertility. The purpose of this study was to evaluate the effects of palcitaxel loaded niosome in combination therapy with B.P extracts to reduce unwanted effects of palcitaxel and enhance fertility in male rat with spinal cord injury.

Methods

Plant Collection and Preparing Extract:

B.P was collected from southern and tropical regions of Kerman province in Iran. The herb dried, and the seeds separated and stored at room temperature.

The ultrasound-assisted aqueous extraction method was used to prepare the *B.P* extract. In brief, 40g of the seeds were completely powdered and dissolved in 300ml of distilled water. Then, it was placed in an ultrasonic machine (Made by Hielschor Company, United States) for 30-minutes at frequencies above 200 kHz. After that, it was filtered and then kept at -20℃ for two hours; the rotary mechanism at 45°C was used for separating the solvent. Finally, it was placed at 45°C for 48 hours to completely separate the solvent. The aqueous extract was dried in the oven, powdered and stored at -20°C until used.

Preparation of niosomes containing Paclitaxel by thin-film hydration method

At First, the fat phase containing a non-ionic surfactant, cholesterol, active ingredient (composed of Surfactant/Cholesterol with the molar ratio of 70:30) was dissolved in 5 ml of chloroform and poured into the balloon. The balloon was connected to the rotary evaporator and the water bath temperature was set at 60±1°C, the balloon rotational speed in 180 rpm consists

of the vacuum pump. This was done for 15minutes to create a thin film on the balloon bottom. Then 7-ml of Paclitaxel in phosphate buffer was added to the balloon to hydration film and form niosomes. The procedure was performed with various surfactants, and the formulations obtained by this method were kept in glass containers for further investigation.

To evaluate quality of the niosomes formation, optical microscopy was used. A drop of formulation was placed on the coverslip and then viewed with a magnification of (40x×10x) and (100x×10x).

Particle Size Distribution and Transmission electron microscopy

In order to investigate the particle size of the niosomes, a Malvern instrument with a laser ray diffraction technique was used. A few drops of niosome suspension were poured into the machine cell containing distilled water. The lens of the machine has the capability of separating the niosomes from 0.1-100 μm. The machine counts and determines about 2000 vesicles in 15 seconds. The machine cell was a flow-through type and after one step of ventilating the machine at 3500-rpm and adjusting the rate of deionized water flow to 1600-rpm, the background was prepared using the Malvern Mastersizer software. Then, the niosomes suspension was added into the machine cell drop by drop. The particle size of each formulation was determined three times using this method.

To investigate the morphology of the niosomes and to confirm particle size, a drop of suspension of niosome particles was placed on carbon film and then it has been imaged after drying at room temperature using a transmission electron microscope (Zeiss, Model EM900; Germany).

A Comparison between the Free Paclitaxel and Paclitaxel-Loaded Niosome using MTT assay

The fibroblast cells (SKM) were obtained from the Pasteur Institute of Iran. SKM cells were cultivated in DMEMα medium (Dulbecco's modified Eagle's medium) containing 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 g/mL) in an incubator at 37℃ with 5% CO2. Cells were plated at the density of 5×103 per well in a 96-microplate well for the MTT assay. Cells were incubated with the different concentration of pure Paclitaxel and PLNs (0, 10, 25, 50, 100 μM). Cell viability and IC50 of niosomal formulations of paclitaxel and the standard drug was estimated. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium reduction colorimetric assay was used to measure the effect of PLNs on SKM cells metabolic activity.

Animals and experimental groups

In this study, 48 male Wistar rats weighing 250±5 g were randomly divided into six groups. Rats were purchased from the animal department of Kerman University of Medical Sciences. Animals were kept in standard light conditions at temperatures of 23±2°C. Rats anesthetized using 25 mg/kg ketamine, 5 mg/kg xylazine and the animal's back hair shaved and disinfected. A cut was made in middle line; the T9 vertebral muscle removed without damaging the dura, then a model of contusion of the spinal cord injury was created by placing a 10 g weight from 25 mm height on the T10 of spinal cord (Heidarizadi, 2017). After the injury, the muscle and skin were sutured, the bladder drain was emptied twice daily and animals were examined for two months (Radojicic, 2007). The first and second groups received 2.5 mg/kg PLN on the calculated release rate of drug on days 3, 7 and 10 after spinal cord injury. 3th day was selected because; locomotion in the groups was measured using the Basso, Bresnahan, and Beattie (BBB) test 48 hours after SCI (BBB score≤3). The second and third groups received 300 mg/kg aqueous extract of B.P daily for 14 days via oral gavage (1-14 days after SCI). In the control group, the normal saline was used. In the sham group, laminectomy was performed without SCI and no therapy was used. Normal control rats were not exposed to SCI or treatment. The weights of the rats were recorded on the first and last day of the experiment.

Examination of Sperm

The rats were anesthetized with intraperitoneal injections of 5 mg/kg xylazine and 50 mg/kg ketamine. The testes from the right side of the rats were removed on the 30th day, and the other testes from the left side of the animals were removed on the 60th day. The testicles of the

animals were weighed, and after that the tail of the epididymis were removed. This area was kept in T6-containing Bovine Serum Albumin (BSA) for one hour in an incubator (37°C). Percentage of sperm motility, the viability of sperms, and ESR were investigated. Testes were prepared for histological assessment, examined by light microscopy and analyzed using motic microscopic software.

Study of gene expression in spinal cord and testis

Total RNA was extracted from the site of the injury in the spinal cord and testis using RNX-plus TM Reagent (Cinnaclon, Iran) according to the manufacturer's protocol on the 30th and 60th days. Then cDNA was synthesized from 1 µg of total RNA and RT-qPCR was accomplished using the primer sequences listed in Table 1. NGF gene expression was checked in spinal cord samples, and *CATSPER1* and *CATSPER2* genes were studied in testis samples.

Table-1 Primer used for this study.

Gene	Sequence(5'-3')
NGF	F:CCTCTTCGGACACTGTGGA
	R:CGTGGCTGTGGTCTTATCT
CATSPER1	F:TCTTGGAGCGATGAGGAC
	R:GACGATTGTGTTCAGGCA
CATSPER2	F:TGGTTGTTGCTTGGTTCC
	R:TTCCTTGACTGGTTCCTCT
Beta- actin	F:GGCATCCTGACCCTGAAGTA
	R:GGGGTGTTGAAGGTCTCAAA

The selected primers were amplified using the SYBR Green Takara Master Kit according to the instructions of the supplier on Rotor-Gene 3000 (Corbett Research, Australia). The initial concentration of each sample was normalized against the β-actin.

Behavioral testing

The BBB test with a score of 0-21 (0:no movement-21:normal movement) was used for mobility evaluation from the test. Evaluation of movement in all groups within the first 48 hours after spinal cord injury, and then recorded once per week for twelve weeks. The final score was reported as the mean. Von Frey Filament test (North Coast Medical, Inc) was used for mechanical testing. It was determined by providing a pressure against the hairless skin of the Rat's hind paws.

Data analysis

Statistical analysis was performed with SPSS Version 26. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was accomplished at the 0.01 and 0.05 level.

Ethical Statement

Ethical guidelines Animal experiments in this project were approved by the Animal Ethics Committee of Veterinary faculty of Shahid Bahonar University of Kerman and followed with the Guidelines for the Care of Laboratory Animals in Research (Animal Ethical Approval Number: IR.UK.VETMED.REC.1400.003).

Results

All steps from plant extraction and Niosomal formulations in animal model are illustrated in the Fig. 1.

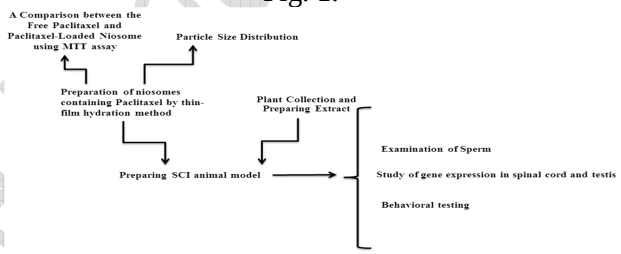

Fig. 1. Schematic representation of the method used in the present study.

Characterization of Niosomes

Niosome structures (MLV, SUV, etc.), (Spherical, tubular, polyhedral, etc.), wall diameter, crystal formation, surfactant and cholesterol content, particle size range, or phenomena such as niosome aggregation were investigated using optical microscopy. The size of the niosomes was approximately 200 nm (Fig. 2a). Compared to the pure drug, the rate of drug trap was equal to 98% and the rate of drug release was equal to 85%. They were calculated after 180 minutes. The drug release study showed that the pattern of drug release from the niosome formulation was slower, and this result indicated higher stability of the niosome formulation.

To investigate the particle morphology and to confirm the particle size, an electron microscope

image was prepared from the optimized sample. Fig 2b showed the existence of spherical vesicles with 200 nm in size. A TEM image of the optimized formulation is shown in Fig. 2b. Based on the TEM image, niosomes have distinct spherical shape with an absolute wall enclosing an aqueous core.

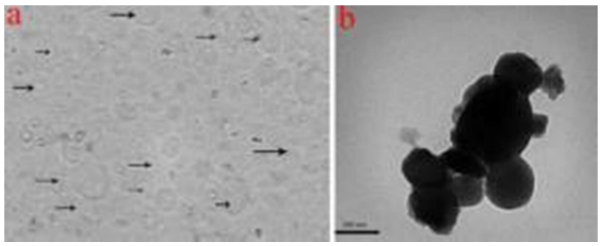

Fig 2- a) Niosomal Vesicles under Optical Microscopy, b) Transmission electron microscopy (TEM) micrographs of niosomes

3.2. MTT test

SKM cells were treated with niosomal formulation and pure drug for 24 hours. Screening of the Cytotoxicity of Paclitaxel using MTT assay demonstrated that the pure drug had more cytotoxicity effect on fibroblast cells with 50% cell death (IC 50) (Fig.3a).

At concentrations of (0, 10, 25, 50, 100 µM) there was a significant difference in cell death and proliferation ($p<0.05$) (Fig.3b).The results show that by increasing the concentration of the Paclitaxel, the viability of the cells decreased compared to the control ($p<0.05$).

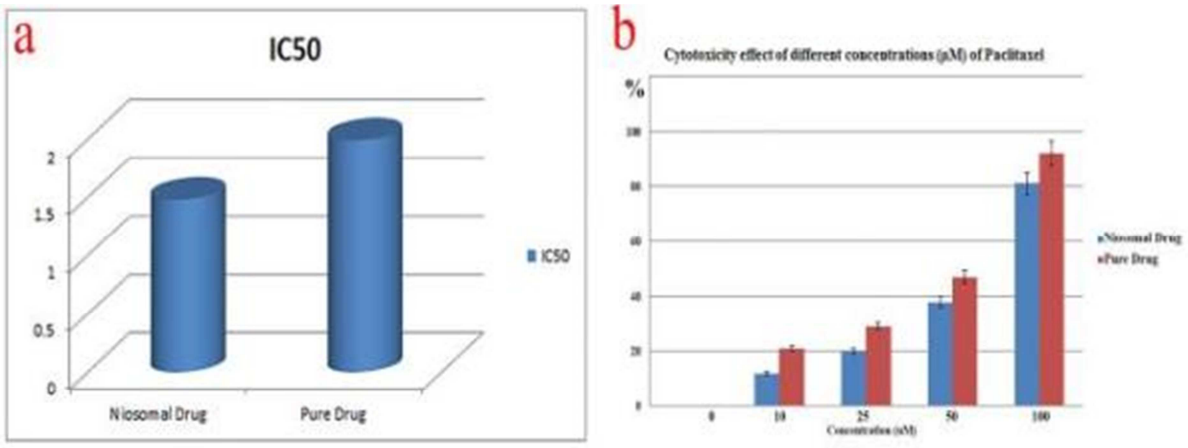

Fig 3- a) Niosomal formulation of Paclitaxel was less toxic than free drug on SKM cells. b) Cytotoxicity effect of different concentrations µM) of Paclitaxel on fibroblast cells determined by MTT assay ($p<0.05$).

Behavioral Tests

The BBB test was used to evaluate the mobility improvement trend. This test is graded from 0-21. These grades range from no hind foot to normal movement. Grades 0-7 related to the evaluation of hip, knee and ankle position, grades 8-13 to evaluate toe position and coordination of movement, and grades 14-21 related to trunk stability, tail position and toe position. Fig 4a shows the mean degree obtained in different groups. This figure shows that the BBB grade in groups 1 and 2 had a significant difference with the 3 and control groups (Fig.4a). The results of the mechanical behavior test measured in the stimulation threshold are presented in Fig. 4b. This result showed a significant difference in the stimulation rate between groups 1 and 2 at the end of day 60 with other groups, but this parameter was not observed in rats of 3 and control groups ($p> 0.05$).

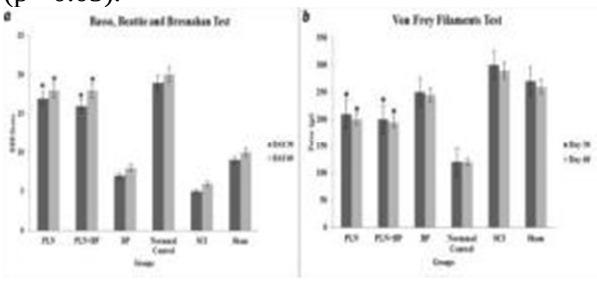

Fig 4- a) There were significant differences between the first and second groups comparing to the others in BBB Test ($p<0.05$), b). Touch sensitization using von Frey filaments There were significant differences between the first and second groups in comparison to the others ($p<0.05$).

Examination of Sperm

The results of sperm motility analysis showed that on days 30 and 60, motility was higher in groups PLNs, B.P, and PLN+B.P than the other groups, but it was only statistically significant (P>0.05) in group PLN+B.P on day 60 (Table 2). Progressive motility was considered in this study, and sperms with low motility were not counted. Sperm viability was significant in the PLNs+B.P group at day 30. On day 60, although sperm viability was high in the PLNs and B.P groups than the others, but it was insignificant (P> 0.05). Epididymal sperm reserves (ESR), testis weight, and body weights were not significantly different in the experimental groups compared to the control group. Besides, the percentage of sperm viability, seminiferous tubules Diameter (µm), and testes epithelium thicknesses (µm) were improved in PLNs+B.P group (Table 2).

Table 2. Effect of different treatments on body weight, testis weight, and sperm parameters (*: P <0.05)

Trait		Body Weight (gr)	Motility (%)	Viability (%)	epididymal sperm reserves×10^6	*seminiferous tubules Diameter* (μm)	testes epithelium thickness (μm)	Testis Weight (gr)
Normal Control	Day 30	251.02± 1.75	28.84±2.68	48.56±2.06	173.03±1.91	189.92±.083	51.23±1.26	1.45±1.01
	Day 60	249.09±1.27	29.02±1.36	47.32±1.85	172.98±1.68	190.36±1.63	49.21±2.01	1.46±1.12
SCI Control	Day 30	248.13±1.94	7.07±0.01	41.25±1.04	168.93±1.71	195.91±2.53	45.35±1.56	1.47±1.89
	Day 60	245.38±2.14	8.22±1.97	42.51±1.09	168.15±2.71	196.36±0.86	43.09±3.24	1.46±1.19
Sham	Day 30	249.21±1.17	18.08±0.80	43.14±1.89	166.39±1.85	193.54±1.27	44.09±3.21	1.47±1.95
	Day 60	245.12±1.86	19.16±1.04	42.86±1.98	165.05±1.28	193.59±1.35	44.96±.092	1.48±1.37
PLNs	Day 30	246.18±1.08	21.07±1.18*	41.08±1.69	171.04±1.25	192.31±2.09	45.68±1.38	1.47±2.10
	Day 60	248.01±1.27	22.98±2.08*	41.96±1.53	172.01±1.38	191.86±1.56	46.09±2.56	1.47±0.91
PLNs + *B.P* extract	Day 30	249.21±1.37	24.08±1.08*	44.04±0.49*	172.24±1.87	191.96±0.94	47.09±2.61	1.46±1.61
	Day 60	250.91±1.16	25.91±1.03*	45.86±1.38*	172.81±2.08	190.61±1.06	48.75±3.01*	1.47±1.92
B.P extract	Day 30	248.08±1.19	20.87±1.08*	41.28±1.29	171.04±1.95	193.92±.083	51.23±1.26	1.46±1.01
	Day 60	248.91±1.07	21.38±2.28*	42.96±1.15	171.21±1.28	192.36±1.63	49.21±2.01	1.46±1.12

The morphology of the testis in animals on days 30 and 60 is shown in Fig. 5. Although significant difference in testis morphology and seminiferous tubule necrosis were observed between treatments, the rate of spermatogonia formation at day 60 in the PLNs+B.P, B.P and PLNs groups was slightly improved. Seminiferous tubule necrosis was increased in SCI and Sham control groups, which decrease spermatogenesis compared to others.

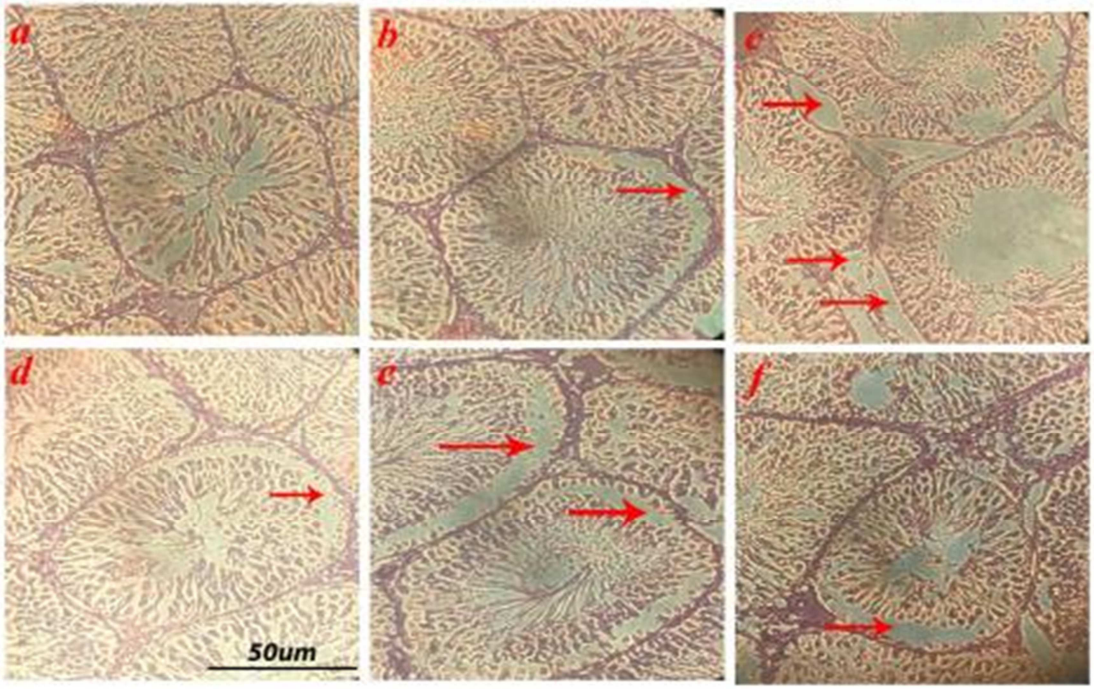

Fig 5- Effect of PLNs and B.P extract on SCI-induced alteration in testis histology. Testis microscopic image of normal group (a), sham control group (b), SCI control group (c), PLN group (d), PLN+B.P group (e) and B.P group (f). Seminiferous tubule necrosis (red arrow), and normal spermatogenesis. Normal spermatogenic cells in the seminiferous tubule are obvious in treated-groups in comparison to disrupted structure of seminiferous epithelia and vacuolization of spermatogenic cells in the seminiferous tubule in SCI group. Testis sections stained with H & E. Images (×100 magnification) are typical and representative of each study group

RT-qPCR

The quantity and quality of the extracted RNAs were checked using spectrophotometry and an OD ratio of A260/A280 for RNA was measured (≈ 2 in optimal conditions). RT-qPCR results of treated animals using niosomal formulation and pure Paclitaxel showed the up-regulation of the CATSPER1 in first, second and third groups comparing to the control on day 30. Its increase was statistically significant in PLNs+B.P group on 30th and 60th days. However, in B.P and PLNs groups, the up-regulation of the CATSPER1 gene was just significant on day 30. ($p<0.05$) (Fig 6a). CATSPER2 gene also showed overexpression in PLNs+B.P, B.P and PLNs groups compared to the control on 30th and 60th days. It was statistically significant in group PLNs+B.P on both 30th and 60th days. In the PLNs+B.P, B.P and PLNs groups, it was statistically significant on 60th day ($p<0.05$) (Fig 6b).

Additionally, RT-qPCR results demonstrated the up-regulation of NGF in PLNs+B.P, B.P and PLNs compared to the others on 30th and 60th days, but its increased was more obvious on 60th day (Fig 6c).

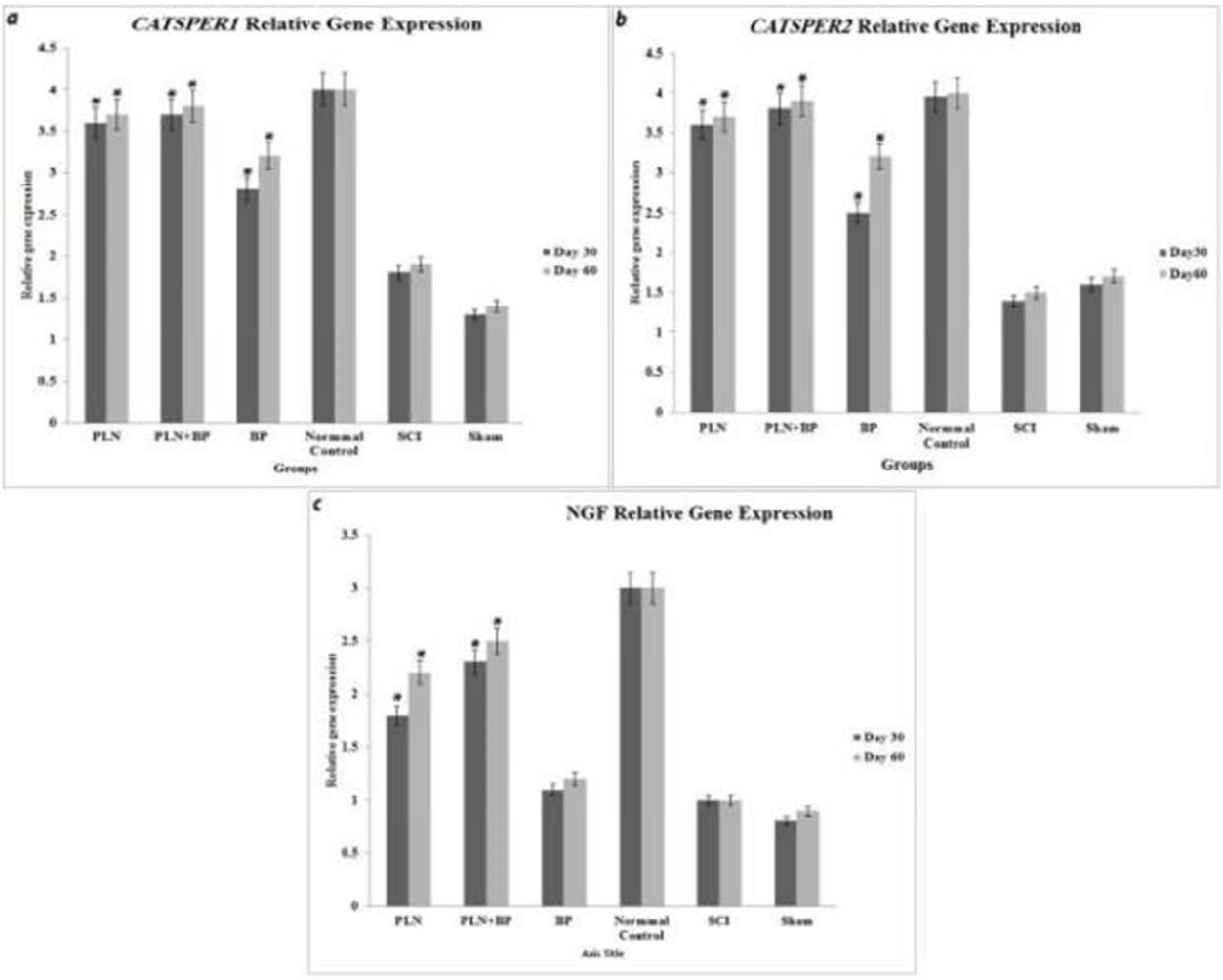

Fig 6- a) Quantitative RT-PCR of CATSPER1 transcripts. Quantification was performed after normalization to β-actin. b)- Quantitative RT-PCR of CATSPER2 transcripts. Quantification was performed after normalization to β-actin. c) Quantitative RT-PCR of NGF transcripts. Quantification was performed after normalization to β-actin ($p<0.05$).

Discussion

The results demonstrated that the combination therapy (PLNs+*B.P*) had the considerable effect on the sperm parameters. Using this novel method effectively declined the pathologic observations in SCI-induced infertility. Besides, *B.P* extract effectively improve sperm parameters in Rats with SCI. It is possible that the mechanisms through which PLNs+ *B.P* illustrated their effects on the male reproductive system by the modulation of endocrine balance and oxidative stress reduction based on the plant ingredients.

SCI male infertility is a problem in global health, and the present study showed considerable support for the protective effect of herbal medicine to increase in the production of sperm motility and reduce the SCI damage to the male reproductive system. Increased motility could result from the antioxidant property of B.P extracts against SCI-induced infertility since B.P extract contains phenolic compounds (Aghaabbasi et al, 2020). The hormonal mechanism for regulation of spermatogenesis based on the herbal medicine is unclear, but it is known that plants have gonadotropic and oestrogenic activities, which classifies the plant as a source of exogenous steroid (Ubah et al, 2021).

The results of the present investigation have indicated that sperm motility was significantly reduced in rats with SCI. Our findings were in accordance with other studies that had reported SCI as a cause of male infertility. Additionally, there were slight reductions in the sperm viability and epididymal sperm reserves in both SCI and sham groups, but they were increased in the treated groups (insignificant).

In the current study, the effect of PLN formulation on spinal cord repair was investigated. Examination of the encapsulation efficiency showed that a considerable amount of the drug was placed into the vesicles. Investigating the pattern of drug release indicates that drug release is relatively slow, and as expected, the use of Niosome as a drug delivery agent had a significant role in slowing drug release. Because the drug needs more time to pass from the phospholipid layers of liposomes. Comparison of the cytotoxic effect of PLN formulation with paclitaxel indicated the greater effect of standard paclitaxel drug on the death of healthy cells. PLNs formulation can interact with these cells because PLNs directly release the drug into the target cell by attaching into the cell's membrane. As well as, Niosomes preserve the drug from being destroyed and protect the patient from the side effects of the encapsulated drug (Antimisiaris, 2021). Paclitaxel acts as a scaffold for nerve cell growth and can help repair nerve damage (Li et al, 2020).

Previous studies demonstrated that after spinal cord total genomic methylation and DNA methyltransferases protein levels reduced in the spinal cord. Study of histone acetylation as a histone modifications indicated its occurrence reduced after SCI while the acetylation of histone 4 increased after peripheral axotomy. If epigenetics modifications such as histone acetylation affects the effectiveness of axon regeneration in the CNS, their manipulation and consumption of different drugs such as paclitaxel can form the basis for novel therapies to regenerate axons in patients with SCI and much more problems such as infertility after SCI (Davaa et al, 2021).

Hong et al, 2020 reported that after SCI epigenetic status in the brain changed and those epigenetic modulations using ascorbic acid may enhance to functional recovery after SCI (Hong et al, 2020). Besides, studies showed that Mesothelin (MSLN) was proved to contribute in paclitaxel administration. Besides, the tumor suppressive function of miR-34a was enhanced by increasing XIST expression and decreased the expression of MSLN and programmed death-ligand 1 (PD-L1) that affects paclitaxel mechanism of action (El Shihy et al, 2020).

The results of the present study also demonstrated that the SCI had the most deteriorating effect on gene expression. NGF gene expression in rats with SCI was increased after Combination treatment. NGF is one of the most important factors in nerve regeneration (Li et al, 2020). Physiological data showed that the animal's movement and sensation improved significantly. Besides, numerous studies have shown the efficacy of plant-derived compounds to improve male infertility. Acacia hydaspica enhanced the diameter of seminiferous tubule, and epithelial height, but reduced the width of tubular lumen, and interstitial space (Afsar et al, 2017). In

another study, it was demonstrated that Achillea millefoliumin increased epithelial thickness sperm motility and tubule differentiation. Additionally, the aged garlic extracts enhanced sperm count, sperm motility (Salahipour et al, 2017). These all together demonstrated the protective effects of the natural ingredients against testicular problems and oxidative stress (Matczak et al, 2018). Meanwhile, B.P is traditionally used to improve male fertility in Iran. Preliminary results of the present study also showed that the B.P increased the sperm parameters and up-regulated the CATSPER1 and CATSPER2 genes. This effect may be due to the presence of linoleic acid (Aghaabbasi, 2020). Linoleic acid as essential fatty acid plays an important role in the synthesis of prostaglandins, and many of the biological reproductive functions of animals fed by the materials containing linoleic acid (Malcicka et al, 2018). Oyelowo et al. (2020) showed that unsaturated fatty acids increase the reproductive function of rodents by increasing the secretion of the sex hormone (Oyelowo et al, 2020).

However, the results of sperm analysis can influence on the evaluation under application of different techniques. Although, the method of evaluating sperm in this test was completely controlled, but sampling under similar conditions can have different results. Despite such limitations, this study is valuable as the first report to study the sperm properties in the treatment with *B.P* extract. The results of the present study support the clinical potency of herbal drugs in men with SCI, especially to improve sperm motility and viability. In order to evaluate the clinical effects of treatment with B.P, randomized controlled tests with large-scale detailed schemes are needed. Additionally, it is necessary to manipulate the miR-34a expression to find the role of PD-L1 and MSLN epigenetic regulation in paclitaxel administration in SCI.

Conclusion

B.P may provide an efficient choice for infertile SCI male with low semen quality. Investigations into the mechanism of action recruited by *B.P* extracts would be necessary to find the medicinal value of this plant thoroughly. Our results confirmed the importance of the effect of PLNs on the sperm parameters, and hence fertility potential. It also demonstrated the importance of combination therapy on sperms quality. Besides, epigenetics effects of paclitaxel are known to offer a mechanism of control the gene expression but combination therapy by using PLNs provides insights to reduce its side-effects and epigenetic modifications that affect many fundamental cellular processes in regenerative response after SCI. However, more studies are needed to study more reliable prediction of combination therapy depending on the extent of SCI or locomotion deficits.

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Conflict of interest

The authors declare no conflicts of interest.

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