Journal of Medicinal Plants Research Vol. 4(14), pp. 1400-1406, 18 July, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.212
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Laminaria japonica polysaccharides on the recovery of rats' spermatogenic function of testis damaged by chronic local ionizing radiation

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Accepted 11 June, 2010

To investigate the degree of recovery by *Laminaria japonica* polysaccharides (LJP) on the damage to the rat spermatogenic function caused by chronic local ionizing radiation, 56 wistar rats were randomly divided into seven groups: Control, three model and three LJP groups. Results showed that although indicators in LJP and model groups were found different from the control group ($P < 0.05$) when compared with the corresponding model groups, except in LJP 7d group the difference of SOD activities was not obvious compared with model 7d group ($P > 0.05$). In the other 7 and 14d LJP group, the SOD and GSH-PX activities increased while MDA content decreased. In addition, mitochondrion membrane potential enhanced in testis cells while DNA injury relieved; furthermore the sperm count and survival rate increased, the difference was significantly ($P < 0.05$) and the testicular tissue damage reduced and mitochondrion were relieved, which were more similar to those of the control group. Our results suggested that lavaged by LJP100 mg·kg^{-1}·d^{-1} at the duration from 7 days before radiation till the end of radiation, LJP has promoting effects on the recovery of the damages to spermatogenic function induced by chronic local ionizing radiation.

Key words: *Laminaria japonica* polysaccharides, ionizing radiation, spermatogenic function, damage recovery.

INTRODUCTION

With the development of nuclear technique, human beings are faced with more dangerous of ionizing radiation than before (Alonzo et al., 2008). As a sensitive organ to ionizing radiation, the testicular injury became one of the most common causes of the dysfunction of spermatogenesis (Zeng et al., 2004; Xue, 2006). In order to prevent and relieve the hazard to human reproductive health, induced by ionizing radiation, avoid the toxicity and the side effects and for the labilization of anti-radiation drugs (Jagetia and Baliga, 2003; Ding et al., 2007), therefore, the seeking for anti-radiation drugs, from traditional food and medicine plant sources, is considered to be a good alternative.

Laminaria japonica is macroscopic algae of plant, belonging to phaeophyta, the phaeosporeae, laminariales, laminariaceae of laminaria (Li et al., 2002), kind of large edible-medicinal seaweed with nutritional value and many health functions (Liu et al., 2007). It also has been used in traditional Chinese medicine for weight loss, detumescence and elimination of phlegm (Guo and Liu, 2008) for almost 1000 years (Li et al., 2005) and was called *kelp* in traditional Chinese medicine. Furthermore it was shown, in several studies, that LJP has not only anticoagulant, anti-arteriosclerosis, anti-tumor, anti-virus, could improve the quality of freezing spermatozoa and other biological properties, but also had anti-radiation effect (Kaeffer et

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Abbreviations: LJP, *Laminaria japonica* polysaccharides; **MDA,** malondialdehyde; **SOD,** superoxide dismutase; **GSH-PX,** glutathione peroxidase; **NMP,** normal melting point; LMP , low melting point; **DMSO,** dimethyl sulphoxide; **EB,** ethidium bromide; **JC-1,** 5,5,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanide iodide; **Δψm,** mitochondrial membrane potential.

al., 1999; Tomohiro et al., 2006; Niu et al., 2008; Hu et al., 2009, 2010). The study in our group indicated that LJP has the effects of anti-fatigue and anti-anoxia, and it also could improve the activity of antioxidase. It also has apparent protection to the injury of rat's testis induced by acute ionizing radiation (Yan et al., 2002, 2003; Ren et al., 2007).

At present, the study of the plants that had protection to spermatogenic dysfunction was concentrated on traditional unity or mixed medical plants such as epimedium, tuber fleece flower root, fructus lycii, desert living cistanche (Li, 2006), but there was no report for the role of LJP in the recovery of spermatogenic dysfunction, induced by chronic ionizing radiation. Current study was to observe the recovery effect of LJP on the rat's spermatogenic function which was damaged by a chronic local ionizing radiation, to explore the potential mechanisms through which LJP could improve the recovery of rat's spermatogenic dysfunction induced by radiation, and to provide the scientific foundation for further study on the antiradiation mechanisms of LJP.

MATERIALS AND METHODS

Plant materials

L. japonica is obtained from dried samples generated in Lianjiang country of Fujian Province, China, and purchased from dried goods market of Wuhan city, and the material was identified by Zhang S.H., Professor of Huazhong Agricultural University. A voucher specimen has been deposited in the library of Public Health School of Wuhan University.

Drugs and reagents

Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) kits were obtained from Jiancheng Bioengineering Institute (Nanjing, China). JC-1 Mitochondrion membrane potential kit was obtained from Bi Yuntian Biology Technological Institute (Shanghai, China). Normal Melting Point Agarose (NMP) and Low Melting Point Agarose (LMP) were obtained from Amresco Company (USA). Tris was obtained from Merck Company (New York, USA). Sarcosine sodium, DMSO, TritonX-100, ethidium bromide were obtained from Sigma Company (USA). Others were analytical pure, obtained from Chemical Reagent of China Medicine Group (Shanghai, China).

Extraction of LJP

The method of Li et al. (2000) was used in the extraction of *L. japonica* polysaccharides. The procedures are described as follows: *first*, dried *L. japonica* were washed to remove the impurities and crushed to become powder and then dissolved by 0.1 mol.L^{-1} HCl solutions to extract fucoidan and laminarin while leaving the alginate in high concentration residues. Solution of Na_2CO_3 (1%) was used to react with alginate which presented in the solution to generate sodium alginate. *Second*, the alginate and laminarin were separated by ethanol in the concentrations of 60 and 85% respectively, because their solution power were different in ethanol. Then what was eluted by DEAE cellulose column respectively, in addition vacuum concentrator and freeze drying was used to extract crude products. The extracted products were: crude sodium alginate, fucoidan and laminarin respectively. The purity was qualificated by a specific rotary power (Li and Dou, 2007) and gel chromatography (Yang et al., 1997) then we mixed them uniformly and determined that the ingredient was single. These products were mixed and stored at 4°C in refrigerator for further use.

Experimental animals

Fifty six 4-week-old male Wistar rats were obtained from the CDC Animal Center in Hubei Province (Wuhan, China). These rats were housed in fourteen groups in rectangular cages (40 × 25 × 20 cm) with wire mesh lids under standardized animal room conditions (12 h light/dark photoperiod at 23°C and 60% humidity). Food in pellets and tap water were available *ad libitum*. Animal experiments were performed according to the guidelines for the care and use of laboratory animals established by Wuhan University (Wuhan, China). These guidelines are in accordance with the Declaration of Helsinki of the World Medical Association (Wang et al., 2002).

Experimental grouping and treatment

In order to explore the spontaneous recovery of rat's spermatogenic function caused by chronic local ionizing radiation and the effect and potential mechanisms of recovery, which was exhibited by the use of *L. japonica* polysaccharides (LJP), of the damaged rat's spermatogenic function caused by chronic local ionizing radiation, so after seven days of adaptive feeding, the male rats were randomly divided into seven groups: radial model 1d, 7d,14d groups and interfered with LJP radial model 1d,7d,14d groups which the rats were killed at (1, 7 and 14) days after the cessation of irradiation, and the normal control group that the rats were killed at the 14^{th} day after the cessation of irradiation in the same manner though without irradiation, and there were eight rats in each group. Then, the groups were named as control group, model 1d group, model 7d group, model 14d group, LJP 1d group, LJP 7d group and LJP14d group, respectively. Because the rats in this study need to be lavaged from 5-week-old, and the weight was lighter at this time, so we adopted 100 $mg \cdot kg^{-1} \cdot d^{-1}$ as intervention dose according to the formerly results of our group (Luo et al., 2004). The rats in LJP 1d, 7d and 14d groups were lavaged with LJP dissolved by physiological saline; the other groups were lavaged with equivalence physiological saline. The model 1d, 7d, 14d groups and LJP 1d, 7d, 14d groups were administrated with ^{60}Coγ partial irradiation on the lower abdomen while the places above the anterior superior iliac crest were covered with 3 mm thick grid except the control group. The radiation source-target distance was 80cm. The total dose was 2.3 $Gy.rat^{-1}$ and the absorbed dose rate was 0.925 $Gy.min^{-1}$ (GWXJ80 ^{60}Co teletherapy machine, Nuclear Power Equipment Manufactory, China).

The study of spermatogenic cell and its mitochondrial structure and injury of spermatozoa on rat

Testicular tissue of 3 mm^3 (1 mm × 1 mm × 3 mm) size was obtained rapidly and fixed with 2.5% glutaraldehyde phosphate solution then we prepared electron microscopic sections. The structure of mitochondrion in testicular cell was observed by means of transmission electron microscope (H-600, Hitachi Company, Japan). A 10% neutral formaldehyde solution was used to fix the testes which were then embedded in paraffin and sectioned into 4 μm sections.

We then performed HE staining and light microscopy on testicular

tissue to compare their morphological changes, using a BX51 microscope camera to take a photograph (Olympus Company, Japan).

The shredded epididymis was transported into 6 ml normal tube with saline, then put in water bath at 37°C for 10 - 15 min. It is dripped into blood count panels and count sperm amount (N) in 5 boxes under microscope (200X). The amount of the sperm was diluted to $N \times 5 \times 10^4$ times per milliliter. Sperm survival rate was determined by using one-step eosin-nigrosin staining technique (Bjorndahl et al., 2003). Sperm that were white or unstained were classified as live, while those that showed pink or red coloration in the head region were considered dead. At least 200 spermatozoa were assessed for each preparation. Sperm survival rate = unstained sperm count / (stained + unstained sperm count) × 100% (BX51 microscope, Olympus, Japan).

The study of oxidative damage of testicular tissue on rat

Testis was separated, taking 1/2 testicular tissue of one side, rinsed with 0.9% sodium chloride solution thoroughly. After measuring, it was made into 10% tissue homogenate with 4°C 0.9% sodium chloride solution, and then centrifuged at 1000 g for 10 min under 4°C (5702 centrifuge. Eppendorf Company, Germany) and the supernatant were packaged. Oxidative indicators, such as MDA, SOD and GSH-PX were determined according to the manual strictly by using the spectrophotometer (722 Spectrophotometer, Shanghai Precision Scientific Instrument Ltd, China).

The study of mitochondrial membrane potential of testicular cell and the injury of DNA on rat

The other 1/2 testis was put into -70°C ultra cold freezer (Haier Company, China) for further use; frozen sections were made according to the manual of JC-1 kit. Eight slides in each group were selected to take photograph by using laser scanning confocal microscope (LSCM) (TCS SP2-AOBS-MP, LEICA Company, Germany). We dealt with each slides by Image-Pro plus 6.0 software according to Muramatsu's method (Muramatsu et al., 2007), collected the data, and indicated the level of mitochondrial membrane potential (Δψm) measured at 590 nm for J-aggregates (red fluorescence) and at 530 nm for J-monomer (green fluorescence). The ratio of 530/590 nm was considered as the relative Δψm value.

Single cell suspension was prepared in the remaining 1/2 testis after the capsule and blood vessel were removed, adjusted cell concentration for 10^6 - $10^7.ml^{-1}$; stored at 4°C in refrigerator for further use. The experiment determined cell survival rate by observing trypan blue stain effect (Zhang et al., 2001). We carried out it with basic alkaline single cell gel electrophoresis empirical method modified by Singh et al (1988), and observed the results by fluorescence microscope, took a photograph synchronism by BX51 fluorescence microscope attached to a solid-state camera (Olympus, Japan). Eight slides were prepared for each treatment. One hundred cells per slide were randomly counted for incidence of comet-like cells and 20 cells measured for tail length. Tailing rate (%) (The percentage of the cells with tail) and tail length (μm) (=maximum total length between comet head and tail−head diameter) were used to assess the DNA damage levels.

Statistical analysis

All results were expressed as mean ± standard deviation for each group. All calculations and statistical analyses were done with SPSS software for Windows version 17.0 (SPSS Inc., Chicago, IL). We could see that all data was normally distribution by explore analysis. Statistical significance in the difference between the means of the control group to model and LJP groups was evaluated by one-way analysis of variance (ANOVA), and between LJP groups and corresponding model groups was evaluated by independent-sample t-test. Significance was set as $P < 0.05$.

RESULTS

LJP on recovery of rats' spermatogenic cells and sperm damaged by radiations

In the control group, we could see spermatogonia, spermatocytes, spermatids arranged compactly and regularly in turn from basal lamina to cavosurface in seminiferous tubules of testis, and there were piles of mature sperm in lumens. Compared with control group, the model groups, stratifications and numbers of spermatogenic cells in rats' seminiferous tubules of testis decreased gradually. The spermatogenic cells were arranged crumbly, and mature sperms in lumens decrease, but there were more cells cast-off; the basilar membrane of seminiferous tubules in model groups crimp intensify, and the cell layer of spermatogenic cells is indiscriminate in model 14 d group, and cavity was formed; the change of LJP groups is similar to the corresponding model groups, but the arrange of the cell layer of spermatogenic cells, cells cast-off in lumens and the number of mature sperm were better than that in the corresponding model groups. The results are shown in Figures 1A-C.

As is shown in Figures 2A-C, from the electron microscope, we could see that there was more mitochondrion in cytoplasm of the control group, and the size of it was almost the same. The structure of spinal meninges was sharp. In eukaryotic cells, oxidation phosphorylation was proceeding mainly on spinal meninges of mitochondrion, and it was the central link of energy metabolism, When radiation injuries was occurred, the mitochondrial matrix would expanse, the spinal meninges would decrease, and the oxidation phosphorylation in mitochondrion would be inhibited, so the normal energy metabolism in cells was affected (Xia et al., 1998).

But in model groups, electron-dense lipid granule could be seen in cytoplasm, mitochondrion in cytoplasm which has differed in size has swelling. Spinal meninges have decreased, cavities have formed and parts of mitochondrion have degenerated; and the mitochondrion has damaged seriously. In LJP groups, there were lipid granules in cytoplasm, the size of mitochondrion almost the same, the structure of spinal meninges was sharp than that of model groups, some of the mitochondrion has swelling, but the injury was slighter than that of the corresponding model groups.

From Table 1 we could see that, compared to the control group, the sperm count has decreased obviously

in model groups and LJP groups, the difference was significant (P< 0.05); compared with corresponding model group, and the sperm count of the LJP groups was higher than

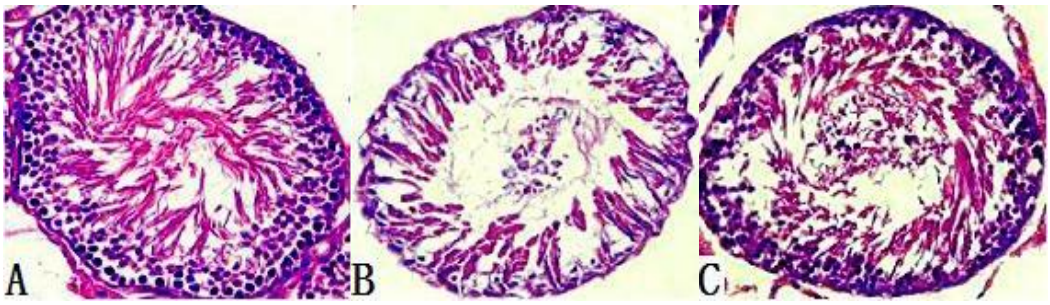

Figure 1. LJP on recovery of rats' testicular seminiferous tubules damaged by radiations. (A) The control group, (H&E staining, ×200). (B) Model 14 d group, (H&E staining, ×200). (C) LJP 14 d group, (H&E staining, ×200).

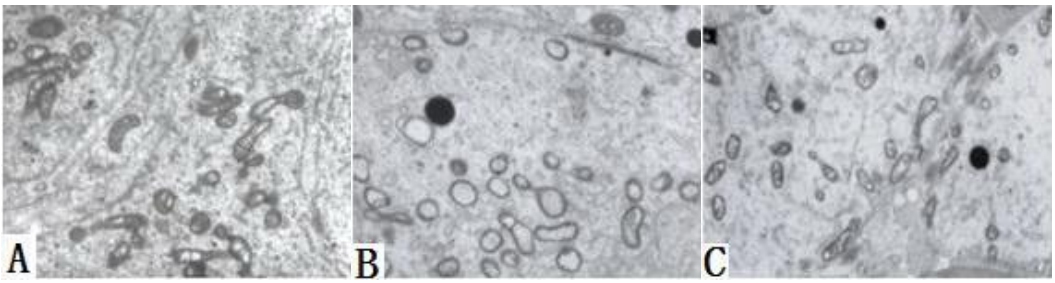

Figure 2. LJP on recovery of rats' testis cells mitochondrion damaged by radiations. (A) The control group, (TEM×4000). (B) Model 14 d group, (TEM×4000). (C) LJP 14 d group (TEM×4000).

Table 1. Effect of LJP on sperm count and survival rate in irradiated rat.

Group	Sperm count (×10^6/ml)	Sperm survival rate (%)
Control	21.60 ± 1.22	74.50 ± 1.25
Model 1d	15.15 ± 0.70*	57.19 ± 3.48*
Model 7d	14.25 ± 0.36*	55.25 ± 3.15*
Model 14d	13.73 ± 0.50*	55.19 ± 3.12*
LJP 1d	20.48 ± 0.50*†	61.97 ± 2.65*†
LJP 7d	19.28 ± 0.50*†	64.75 ± 4.12*†
LJP 14d	18.75 ± 0.48*†	69.75 ± 2.70*†

Note: n = 8 (in each group)); (mean ± S.D); *: P<0.05 compared with control group; †: P < 0.05 compared with corresponding model group.

the corresponding model groups (P < 0.05). The tendency of survival rate of sperm and its count were identical in model groups, but in LJP groups, it was opposite. The differences between the model groups, LJP groups and the control group were all statistically significant (P < 0.05).

LJP on recovery of rats' testicular tissue oxidation damaged by radiations

As shown in Table 2, the content of MDA in model groups and LJP groups was obviously higher than the control group (P < 0.05); and it obviously has decreased more in LJP groups than in corresponding model groups (P < 0.05). Compared with control group, the activity of SOD in model groups have decreased obviously (P < 0.05), but in LJP 1d group, the activities of SOD have increased (P<0.05), and in LJP 7d, 14d groups, the activities of SOD have decreased obviously (P < 0.05); The activities of

SOD were increased activity in LJP 1d, 14d groups compared to corresponding model groups (P < 0.05). The activity of GSH-PX in the model groups and LJP groups have significantly decreased compared with control group (P < 0.05); but were obviously higher in LJP groups compared

Table 2. Effect of LJP on MDA, SOD and GSH-PX in irradiated rats' testicular tissue.

Group	MDA (nmol/mgprot)	SOD (U/mgprot)	GSH-PX (U/mgprot)
Control	1.30 ± 0.27	155.11 ± 4.49	31.76 ± 1.76
Model 1d	2.42 ± 0.42*	126.18 ± 6.15*	17.08 ± 1.22*
Model 7d	2.16 ± 0.34*	123.98 ± 11.38*	17.68 ± 1.71*
Model 14d	2.24 ± 0.16*	124.46 ± 5.32*	16.99 ± 1.96*
LJP 1d	1.85 ± 0.08*†	173.01 ± 9.45*†	26.78 ± 1.12*†
LJP 7d	1.70 ± 0.09*†	133.54 ± 8.89*	22.90 ± 0.52*†
LJP 14d	1.64 ± 0.35*†	139.97 ± 12.54*†	29.33 ± 1.52*†

Note: n = 8 (in each group)); (mean ± S.D); *: P < 0.05 compared with control group; †: P < 0.05 compared with corresponding model group.

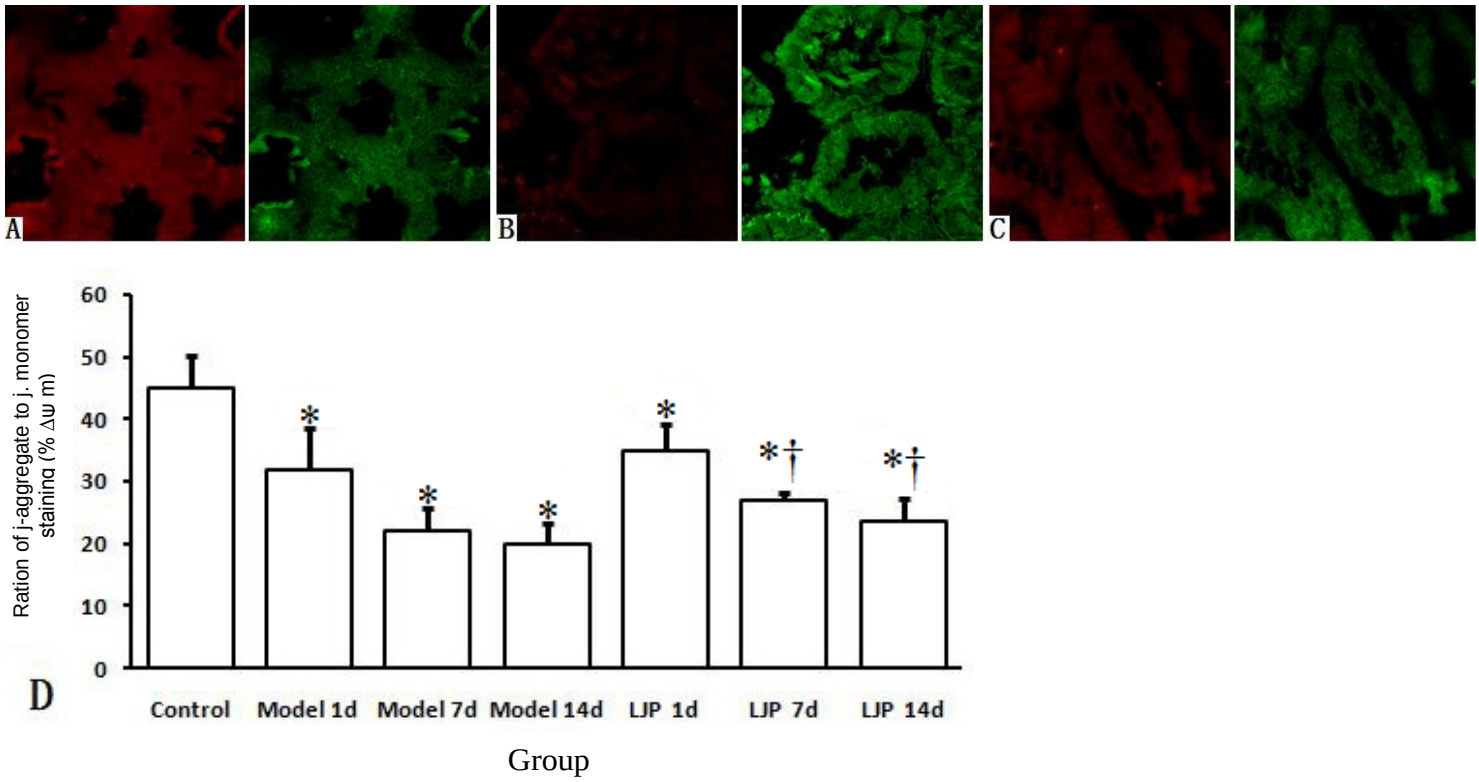

Figure 3. LJP on recovery of rats' testis cells mitochondrial membrane potential (Δψm) damaged by radiations. (A) The control group, (JC-1 staining ×100). (B) Model 14 d group, (JC-1 staining ×100). (C) LJP 14 d group, (JC-1 staining ×100). (D) Comparison of Δψm in each groups; n = 8 (in each group), (mean ± S.D); *: P < 0.05 compared with control group; †: P < 0.05 compared with corresponding model group.

to the corresponding model groups (P < 0.05).

LJP on recovery of rats' testis cells mitochondrial transmembrane potential and DNA damaged by radiations

Detected by JC-1 staining, as it was shown in Figure 3 (A-D), compared with control group, mitochondrial membrane potential (Δψm) of testis cell has decreased a significant difference among them (P < 0.05). In LJP obviously in model groups and LJP groups, and there was groups, however, Δψm was higher than the corresponding model groups, and was obviously higher in

LJP 7, 14d groups than that in model 7, 14d groups (P<0.05). We used fluorescence microscope to observe single cell gel electrophoresis, the results were shown in Figure 4(A-E), in control group, we could see tadpole shape sperm head, and other testis cells were all with round fluorescent head, which indicating no DNA breakage. In model groups and LJP groups, we could see the breakage of DNA in some

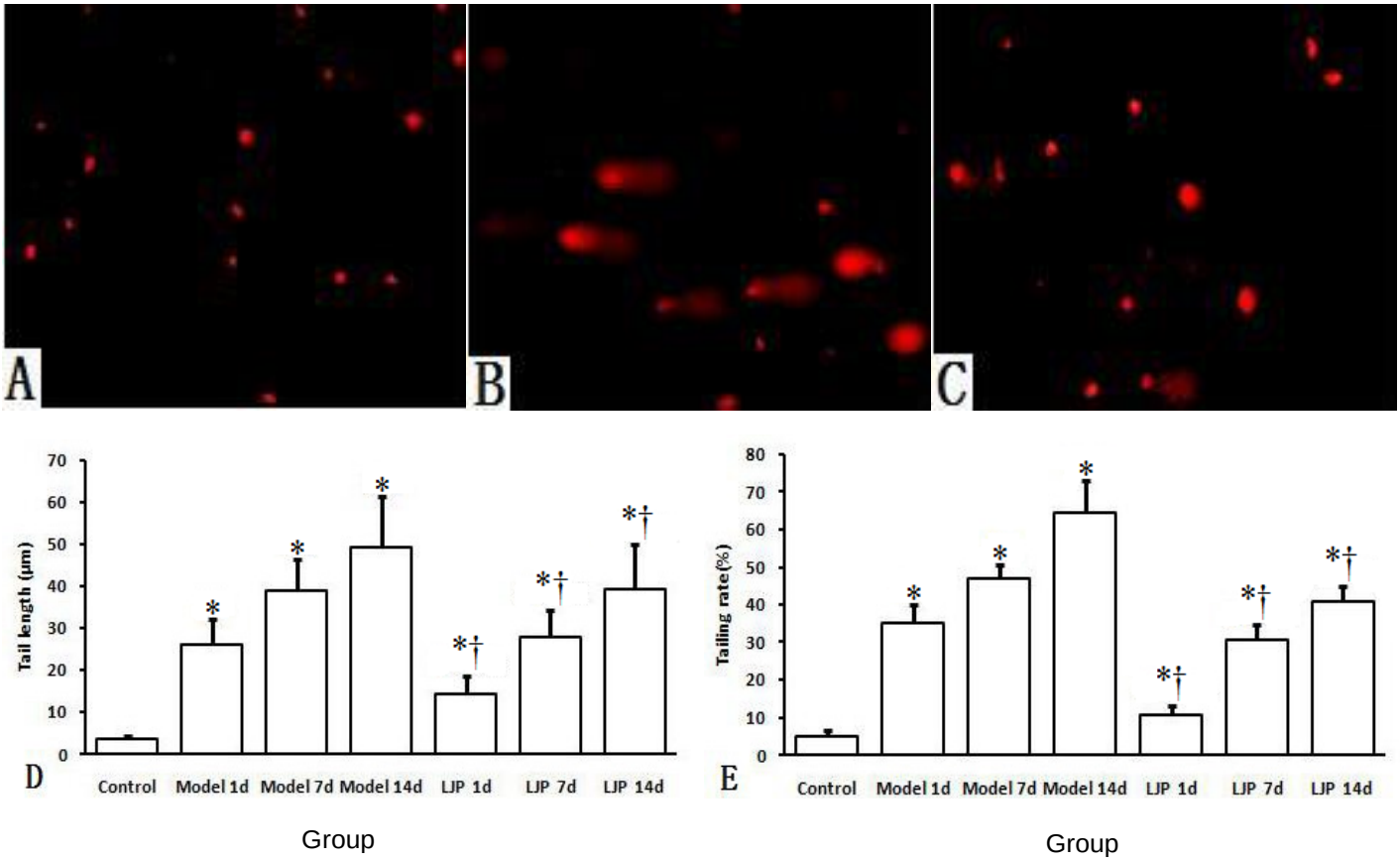

Figure 4. LJP on recovery of rats' testis cells DNA damaged by radiations. (A) The control group, (EB staining ×400). (B) Model 14 d group, (EB staining ×400). (C) LJP 14 d group, (EB staining ×400). (D) Comparison of comet tail length in each group; (E) Comparison of comet tailing rate in each groups; n = 8 (in each group), (mean ± S.D); *: P < 0.05 compared with control group; †: P < 0.05 compared with corresponding model group.

of testis cells, the fragment moved to anode, the middle part of the head showed dense but the periphery light, and there was spindle-shaped or radiated Comet tailing phenomenon, symptomatizing DNA damage. With the cessation of radiation time prolonging, the number and length of the tailing testis cells were all increasing obviously in model groups and LJP groups compared to control group (P<0.05), but were decreasing in LJP groups compared to model groups (P<0.05).

DISCUSSION

As is known to all, *L. japonica* is a plants resource for both food and medicine. LJP is an important functionality component, extracted from *L. japonica*; although Skoryna, Harrison, Fan, Li et al studied the anti-radiation function of it (Fan and Chen, 1988; Zhang and Zhang, 1999; Li et al., 1999, 2002), but there were scarce reports on LJP on recovery of damage by chronic local of ionizing radiation. Our study is showing that in 14 spontaneous recovery days of rat, after the cessation of radiation, the anti-oxidant system of testis has been destroyed and the recovery was slow in the model groups, however, with the recovery time prolonging, in LJP groups, slowed down the lipid peroxidation of testis tissue produced by chronic radiation effectively, enhance the activity of antioxidase, improve the recovery of anti-oxidant defense system, and it was accordance with previous reports that LJP had the effect of anti-lipid peroxidative (Yan et al., 2003; Zhu et al. 2008), and it was also concerned with protecting the spermatozoa quality by reducing the oxidative damage of spermatogenic organ (Chitra et al., 2003).

Our study is also showing that, at the recovery stage of radiation injury in each model group, the mitochondrial membrane permeability of testicular cell on rat

continuously increased, which led to the inflation of mitochondrial matrix, aggravation of the mitochondrial structural injury, and the recovery was unobvious, in addition; the injury of DNA aggravated gradually. This result was match with the conclusion that the injury of mitochondrion would lead to the energy disturbance in the cell and then induced the biosynthesis block of DNA (Xia et al., 1998). And it caused the apoptosis and necrosis of

testis cells, the injury of seminiferous tubules became more serious and finally the number and survival rate of spermatozoa decreased. Although the decrease in the mitochondrial membrane potential, aggravation of the injury of DNA, seminiferous tubules and mitochondrion with time prolonging in LJP groups; but still less than those in the corresponding model groups (Figures 1 - 4). which showed that LJP could amend the mitochondrial membrane potential, release the injury of mitochondrion, what followed next, it promoted the recovery of DNA in testis spermatogenic cells of the rat, slowed down the damage of spermatogenic cells in convoluted tubule, maintained the spermatogenic function of testis, and raise the number and survival rate of sperm (Table 1).

Conclusions

From above we can draw a conclusion that in the recovery stage, at the cessation of radiation for 14 days, the damage of rat testicular tissue continued, the spontaneous recovery of spermatogenic function unobvious, lavaged with LJP100mg·kg^{-1}·d^{-1} to rat from 7 days before irradiation to the end of irradiation could release the injury of testicular tissue on rat s and promote the recovery of spermatogenic function in rat's testis. The mechanism concerned not only with that LJP could enhance the ability of antioxidase, release the DNA damage of spermatogenic cells and the oxidative injury in testis tissue, improve the recovery of anti-oxidant defense system, but also that LJP could promote the upswing of mitochondrial membrane potential, release the injury of mitochondrion, ensure the normal energy metabolism of testis cells, and promote the recovery of DNA injury in spermatogenic cells.

ACKNOWLEDGEMENTS

This work was supported by the Food Nutrition and Education Fund of DANONE Institute (DIC2007-06, China). We are grateful to Dr. You Lu and Chen Chuang (Wuhan University) for their revision and comments on the manuscript.

REFERENCES

Alonzo F, Hertel-Aas T, Gilek M, Gilbin R, Oughton DH, Garnier-Laplace J (2008). Modelling the propagation of effects of chronic exposure to ionising radiation from individuals to populations. J. Environ. Radioact., 99: 1464-1473.

Bjorndahl L, Söderlund I, Kvist U (2003). Evaluation of the one-step Eosin–Nigrosin staining technique for human sperm vitality assessment. Hum. Reprod., 4: 813-816.

Chitra KC, Rao KR, Mathur PP (2003). Effect of experimental varicocele on structure and function of epididymis in adolescent rats: a histological and biochemical study. Asian J. Androl., 5: 203-208.

Ding GR, Guo GZ (2007). Advances in research of radioprotectant. J. Radiat. Res. Radiat. Process, 25: 321-324.

Fan MF, Chen QH (1988). Isolation, analysis and biological activities of sodium alginate. J. China Pharm. Univ., 19: 279-281.

Guo R, Liu YH (2008). The health effects of laminaria japonica polysaccharides and prospect of its application in occupational protection. Int. Med. Health Guid. News, 14: 118-121.

Hu JH, Li QW, Li G, Jiang ZL, Bu SH, Yang H, Wang LQ (2009). The cryoprotective effect of trehalose supplementation on boar spermatozoa quality. Anim. Reprod. Sci., 112:107-118.

Hu JH, Zhang SS, Li QW, Jiang ZL, Zhang DF (2010). Effects of Laminaria Japonica Polysaccharide (LJP) on Boar Spermatozoa Quality Following Freezing-thawing. Anim Reprod Sci. Accepted Manuscript.

Jagetia GC, Baliga MS (2003). Treatment of mice with a herbal preparation (mentat) protects against radiation-induced mortality. Phytother. Res., 17: 876-881.

Kaeffer B, Benard C, Lahaye M, Blottiere H M, Cherbut C (1999). Biological properties of ulvan, a new source of green seaweed sulfated polysaccharides, on cultured normal and cancerous colonic epithelial tells. Planta Med., 65: 527- 531.

Li B, Dou M (2007). Studies on detection of trehalose of yeast extract. Chem. Ind. Times, 21: 47-49.

Li DY, Wang HB (1999). Studies on radioprotection by fucoidan and alginate from Laminaria japonica aresch. J Wuhan Food Ind. Coll., 2: 18-21.

Li D Y, Xu X B, Xiong L, Yin Y M, Yang J, Zhang S H (2002). Radioprotective effects of fucoidin extracted from brown seaweeds. Food Sci., 23: 282.

Li L, Luo Q, Zhang SH (2000). Studies on the classified extract, Identification and physical and Chemical Properties of Laminaria Japonica Polysaccharides. Food Sci., 21: 28-32.

Li N, Zhang Q B, Song J M (2005). Toxicological evaluation of fucoidan extracted from Laminaria japonica in Wistar rats. Food Chem Toxicol., 43: 421-426.

Li XL (2006). Research Development of Antitumor Drug's Damage to Seminiferous Function and Correspond Proteets. Contin. Med. Educ., 20: 50-53.

Liu SL, Wang CY, Wang H (2007). Utilization and Development of Laminaria japonica in China. Food Drug, 9: 34-36.

Luo Q, Wu XM, Yang ML, Huang X L, Yan J (2004). Relation of antiradiation effect of Laminaria japonica polysaccharides and lymphocyte apoptosis. Acta Nutr Sinica., 26: 471-473.

Muramatsu Y, Maemoto T, Iwashita A, Matsuoka N (2007). Novel neuro-protective compound SCH-20148 rescues thymocytes and SH-SY5Y cells from thapsigargin-induced mitochondrial membrane potential reduction and cell death. Eur J Pharmacol., 563: 40-48.

Niu W, Zhao YF, Xu CS (2008). Adavances in research of biological active on polysaccharides of Laminaria. Sci. Tech. Information, 22: 343.

Ren SC, Luo Q, Yang ML, Yang JJ, Yan J, Li ZN, Wang LH, Cui XY (2007).Effect of Laminaria japonica polysaccharides (LJP) on radiation damage of testis tissue in male rats. Chin. J. Radiat. Media Protect, 27: 540-543.

Singh NP, McCoy MT, Tice RR, Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp., Cell Res., 175: 184-191.

Tomohiro O, Jynji Y, Takashi Y, Noriyuki Y, Makoto N (2006). Two fucoidans in the holdfast of cultivated Laminaria japonica. J. Natl. Med., 60: 236-239.

Wang YR, Zhao H, Sheng XS, Gambino PE, Costello B, Bojanowski K (2002). Protective effect of fructus lycii polysaccharides against time and hyperthermia-induced damage in cultured seminiferous epithelium. J. Ethnopharmacol., 82: 169-175.

Xia SX, Chen JP, Jin CZ, Wei K, Zhou PK (1998). Radiobiology. Military Medical Sciences Press, Beijing. pp. 147-149.
Xue SL (2006). The effection of environment factor on male fertility. J Lanzhou Univ (Med. Sci.). 32: 93-96.
Yan J, Li L, Tan XD, Luo Q (2002). Study of the influence of Laminarin on arterial blood gas of anoxic mice. Hubei J. Prev. Med., 13: 3.
Yan J, Luo Q, Yang ML, Wang CH (2003). Anti-lipid peroxidation effect of the Laminaria japonica polysaccharide. Med. J. Wuhan Univ., 24: 219-221.
Yang XT, Mi K, Yang QY, Zhu XY, Sun HZ (1997). Isolation and identification of the polysaccharide LeBD1-1 from the mycelia of lentinulaedodes. Acta Microbiol. Sinica., 37: 119-123.

Zeng GY, Ren DQ, Guo GZ (2004). Radiation injury prevention and treatment. Fourth Military Medical University Press, Xi an, pp. 48.
Zhang Z, Zhang J (1999). The physiological activity polysaccharides in Laminaria japonica. Food Sci. Technol., 3: 52-53.
Zhang ZZ, Heng ZC, Zhao R, Liao Y (2001). The Establishment of Comet Assay for Male Reproductive Cells in vitro. J. Health Toxicol., 15: 99-101.
Zhu HB, Wang CH, Deng QS (2008). Protective effects of Laminaria japonica polysaccharide on carbon tetrachloride-induced liver injury in mice. J. Yantai Univ. (Nat. Sci. Eng.), 21: 204-208.