

Original Article

The effect of date palm seed extract as a new potential radioprotector in gamma-irradiated mice

ABSTRACT

Objective: Date palm seed extract (DPSE) has various compounds revealing antioxidant features. This study aimed to evaluate the radioprotective effect of DPSE in total body gamma irradiation.

Materials and Methods: At first, chemical characteristics of DPSE were analyzed by ultraviolet, visible and Fourier transform infrared spectroscopy. Then, the toxicity of DPSE was assessed. For this purpose, 60 mice were divided into five groups, and each of the groups were injected by the doses of 100, 200, 300, 400, and 500 mg/kg, respectively. At the termination of the experiment, mortality rate and weight loss of all mice were evaluated over a period of 30 days. Finally, the radioprotective effect of DPSE was evaluated by dividing 36 mice into three groups: control, test, and placebo and then were irradiated by Cobalt-60.

Results: According to the findings, there was no mortality due to DPSE. Furthermore, for the maximum dose of 500 mg/kg, the number of mice surviving at the termination of the experiment with and without injection of DPSE was reported as 83% and 41%, respectively. In addition, a significant difference was obtained between radiated mice with and without DPSE injection ($P = 0.035$).

Conclusion: The findings showed that DPSE injected into mice before irradiation has no toxicity and could protect mice from lethal effects of total body irradiation. The use of DPSE as a new radioprotector agent in the human needs further studies, particularly clinical trials.

KEY WORDS: Date palm seed extract, gamma irradiation, radioprotection, total body irradiation, toxicity

INTRODUCTION

Radioprotective agents are compounds which significantly reduce cell damage effects of radiation in human and/or animals, if they were prescribed only before exposure to radiation. These agents can reduce the adverse effects of radiation over cell through one or more mechanisms including: scavenging of free radical, repairing by hydrogen donation to target molecules, formation of mixed sulfides, delaying of cellular division, induction of hypoxia, increasing of cytokines production, evaluation of level of DNA repair, and inhibition of protease in cell.^[1]

The use of herbs in medicine has a long history. Recently, the protective features of various herbs have been studied. Most of these studies have been conducted in Asia, due to the climate, geographical location and importance of herbal medicines used in traditional medicine of native residents.^[2] Compounds having antioxidant properties can have

the radioprotection effect; hence, the herbs that have the antioxidant properties such as scavenging of free radicals, inhibiting of lipid peroxidation, and increasing of glutathione levels in the tissues will have significant radioprotective effect.^[3]

Palm is an important herbal product because it has minerals, sugars, vitamins and antioxidant compounds with high nutritional value growing in different places.^[4] Several studies have shown that date would be a good source of natural antioxidants for medicinal and commercial purposes. Date palm seed extract (DPSE) also contains useful compounds such as phenolic compounds, tocopherols, and sterols.^[5] Several studies have shown that DPSE has antioxidant, antimicrobial, antiviral, and anticancer

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hypoglycemic properties.^[6] Furthermore, it was proved that DPSE possesses the high value of flavonoids. In a study, it was shown that the value of flavonoids was 3102–4430 mg/100 g of DPSE.^[7] Flavonoids have good antioxidant activity; as in the chemical structures of these components, there are catechol and hydroxyl groups which can participate in the oxidation process and remove free radicals.^[8]

To the best of our knowledge, there is no study on evaluation of the radioprotector effect of DPSE in gamma irradiation. On the other hand, as mentioned, there are high antioxidant properties for DPSE. These reasons were the main motivations for this study to evaluate the effect of DPSE as a new potential radioprotector in gamma-irradiated mice.

MATERIALS AND METHODS

At first, chemical characteristics of DPSE were analyzed by ultraviolet-visible (UV-Vis) and Fourier transform infrared (FT-IR) spectroscopy. Then, the toxicity of DPSE was assessed for various doses of DPSE. At the termination of the experiment, mortality rate and weight loss of all mice was evaluated over a period of 30 days. Finally, the radioprotective effect of DPSE was evaluated; as animals were divided into three groups (control, test, and placebo) and then were irradiated by Cobalt-60.

Preparation of date palm seed extract

Palme date seed of Iranian Kabkab species was propped and rinsed with hot water and alcohol, then grinded with industrial mortar. Powder of date palm seeds was dissolved in 1:10 of 100% ethanol to obtain a normal saline solution. For better dissolution, 2W/cm² continuous ultrasound intensity of sonic aid was applied to the solution for 5 min.

Determination of date palm seed extract characteristics

The authentic standards and sample solution were scanned on a computer-controlled UV-Vis Alpha-1860S/1900S spectrophotometer (LAXCO Inc., USA) in range 250–700 nm. Furthermore, FT-IR spectra for DPSE was obtained in the range 4000 to 400 cm⁻¹ with an FT-IR spectrophotometer (Perkin Elmer Inc., USA); as FTIR spectroscopy was used to characterize the structure of flavonoids in the date oil.

Toxicity evaluation of date palm seed extract

After analysis of DPSE characteristics by UV-Vis and FT-IR spectrometry, the acute toxicity of DPSE was determined according to Prieur *et al.* and Ghosh method.^[9,10] Sixty male Swiss albino mice weighing between 27 and 32 g under the controlled conditions of temperature (23°C ± 2°C), humidity (50% ± 5%), and light (10 and 14 h of light and dark, respectively) were divided into five groups and each of the groups were intraperitoneally injected with certain doses of 500, 400, 300, 200, and 100 mg/kg, respectively. Immediately after injection, the mice were fed with adequate food and water. In this test, the measure of toxicity was based on the

mortality and the weight loss of the mice over a period of 30 days.

Evaluation of radioprotective effect of date palm seed extract

To investigate the protective effect of DPSE, another 36 Swiss albino mice were divided into 3 groups; Group A: Without DPSE + with solvent ethanol + normal saline-with exposure (control group), Group B: With DPSE (500 mg/kg and half an hour before irradiation)-with exposure (test group), and group C: With solvent ethanol + normal saline-without irradiation (placebo group). As mentioned, a maximum dose of 500 mg/kg of DPSE was selected to evaluate the radioprotective effect. Our rationale for selection of this dose was based on the fact that by increasing the concentration of radioprotective agent in living cells and organs, higher protective effect will be achieved.

Gamma irradiation

According to the previous studies, the LD_{50/30} doses with low LET (gamma or X-ray) for the Swiss albino mice were ranged from 6 to 7.4 Gy. Hence, a dose value of 7.5 Gy was used to evaluate the radioprotector effect of DPSE in this study. Half an hour following injection of DPSE, the animals were irradiated with a cobalt-60 gamma radiation source (Theratron 780, AECL, Ontario, Canada) with a single radiation exposure of 7.5 Gy. The dose rate of Co-60 machine was noted as 1 Gy/min. To keep the mice under the radiation field, they were placed inside a circular plexiglas phantom with 10 radial sectors that designed a 1 cm plexiglas upper door and hence that they were irradiated as total body. Figure 1 shows irradiation of the animals within the plastic phantom.

Statistical analysis

The results were analyzed using SPSS 11.5 software (SPSS, Chicago, IL, USA). $P \leq 0.05$ was considered statistically significant. Chi-square test was used to determine statistical significance between two groups (irradiated animals with and without DPSE). Furthermore, survival analysis (Log-rank test) was used to obtain a time-dependent survival curve as well as to make comparison between the three study groups (control, test, and placebo groups).

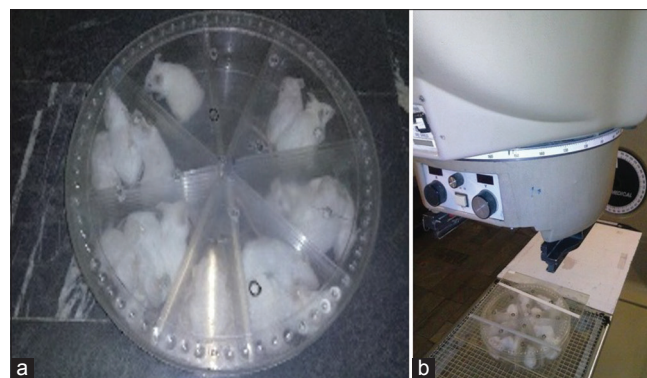


Figure 1: (a) The built plexiglas container to keep the mice under the radiation field. (b) Image of mice under the gamma radiation using Cobalt-60 machine with dose rate of 1 Gy/min

RESULTS

Figure 2 shows the UV–Vis absorption spectroscopy of date oil. It is notable that the absorption peak at 280 nm and 490 nm attributed to the presence of the flavanol and coloured compounds (such as b-carotene and chlorophyll in date oil), respectively.^[11]

Figure 3 demonstrates a typical FTIR spectrum recorded for the flavonoids in the date oil. As it is observed from the figure, there are absorption bands at 1626, 3422, 1070, and 1629 cm^{-1} which relate to C = O stretching of the carbonyl groups, O–H stretching mode of flavonoids and C–O stretching, and C = C stretching, respectively.^[12]

In relation to the toxicity assessment of DPSE, the finding showed that there was no mortality due to DPSE. Although in the first two days, physical activity and the weight of the animals were reduced to 4 g on average, but after that time, they returned to the normal state and over the period of 30 days, no death was observed even at the maximum dose of 500 mg/kg.

The findings related to the radioprotective effect of DPSE showed that for the maximum dose of 500 mg/kg, the number of mice surviving at the termination of the experiment with proposed DPSE value and without DPSE injection was reported as 83% and 41%, respectively. Table 1 shows the mortality rate of different groups.

Finally, the results of Chi-square test revealed that there is a significant difference between irradiated mice with and without DPSE ($P = 0.035$). Furthermore, the time-survival curve for the three groups (control, test, and placebo) using Log-rank analysis is illustrated in Figure 4. The results related to log-rank test showed that DPSE significantly reduced the mortality rate.

DISCUSSION

In this study, the effect of DPSE as a new potential radioprotector in total body gamma irradiation was evaluated. In addition, the toxicity of this radioprotector was investigated.

In recent years, a lot of efforts have been carried out to use the herbal sources of bioactive compounds for controlling certain chronic diseases such as diabetes, obesity, gastric cardiovascular diseases, and cancer. Oxidative stress is one of the most common causes of most diseases. It is an unbalance between the biological defenses and the generation of free radical agents. Ionizing radiation-induced cell damages can lead to a lack of balance between formation and neutralization of free radicals.^[2,13] DPSE can inhibit free radicals and prevent molecular damage mutagenesis.^[14,15] Chemical analysis on the DPSE in this study and other reports showed that it has useful compounds such as phenolic compounds, tocopherols, and

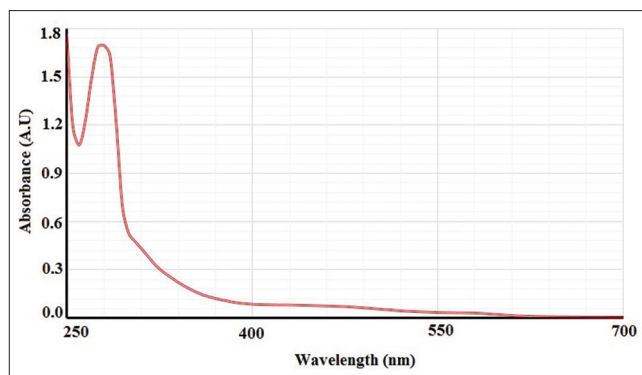


Figure 2: Fourier transform infrared spectrum obtained for date palm seed extract

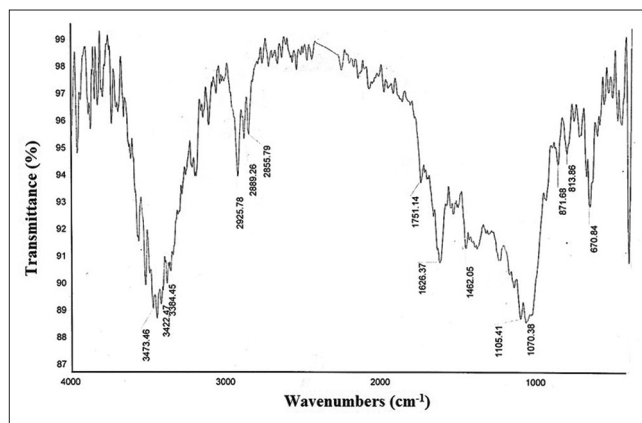


Figure 3: Ultraviolet visible spectra of date palm seed extract scanned between 250 and 700 nm

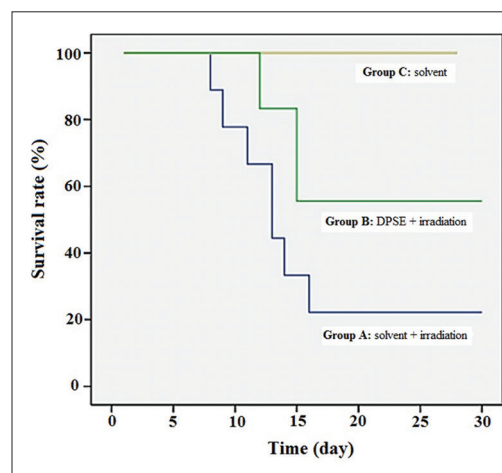


Figure 4: Time-survival curve for control, test and placebo groups using Log-rank statistical analysis

sterols.^[6] Some antioxidant compounds in the DPSE include hydroxytyrosol, gallic acid, caffeic acid, pi-coumaric acid, and oleuropein.^[5,16,17] Phenolic compounds are a group of aromatic plant metabolites that show numerous biological effects such as antioxidant and antibacterial activities. Antioxidant activity of phenolic compounds in plants is mainly due to

Table 1: Radioprotective effect of date palm seed extract on survival of the mice with and without gamma radiation during a period of 30 days

| Group | Mortality in different groups during days | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Number of survivor | Survival rate (%) | | |
|---------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--------------------|-------------------|----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | | | | |
| Test | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 10 | 83 |
| Control | - | - | - | - | - | - | 1 | 1 | - | 1 | - | 2 | 1 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 5 | 41 |
| Placebo | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 12 | 100 |

oxidation-reduction properties of their chemical structures which have an important role in neutralizing the free radicals. Flavonoids are the largest group of natural phenols, and DPSE is a rich source of phenols with 3.43 g/100 g concentration and antioxidants with 0.8 g/100 g concentration.^[18]

In this study, the intraperitoneal injection with 10% ethanol DPSE did not show any mortality during 30 days. There was also no significant difference in mice weight before and after injection of DPSE over the period of 30 days. Investigations showed that the date seeds do not have any toxic effects.^[19]

The overall mortality in the control group was 7 from 12 mice and this occurred in the second week. It may be due to the gastrointestinal syndrome induced by radiation. The main cause of death from this syndrome is that population of intestinal crypt cell proliferation decreases and consequently reduces the epithelial. According to findings [Table 1] and comparing the test group with the control group, it is observed that when using of DPSE, survival rate increased by 42%. In addition, the results revealed that there is a significant difference between radiated mice with and without DPSE; as DPSE significantly reduced the mortality rate. In a study, Habib and Ibrahim evaluated the protective effect of date pits in reducing the oxidative stress injury in rat tissues. They concluded that it decreases the levels of malondialdehyde.^[20] In another study, Basuny *et al.* showed the role of date pits on lipid per oxidation of meat. Their findings revealed that the date pits extract had remarkably the highest levels of total polyphenols and antioxidant activity. Furthermore, the obtained results indicated that phenolic compounds in date pits of khalas variety had high antioxidative effect in reducing the formation of hydroperoxides during storage.^[16] However, the exact mechanism of radioprotective effect of DPSE is not clearly known yet; but according to the chemical derivation of DPSE that consists of rich antioxidant agents, it seems that the dominant mechanism is the properties of free radical scavenging.

One limitation of this study was that the radioprotective effect of DPSE was evaluated only in a concentration (500 mg/kg). Another limitation was the time interval between injection and irradiation; as only a specific interval (half an hour) was used. Finally, only a dose rate (1 Gy/min.) and beam energy (Co-60 machine) were used in this study.

For future research, it is suggested that the radioprotector effect of DPSE be assessed in different concentrations of

DPSE. Furthermore, the evaluation of various time interval between injection and irradiation is proposed as a new work. Furthermore, the use of different dose rates and beam energies can be suggested in future studies.

CONCLUSION

In this study, the radioprotector effect of DPSE was assessed in gamma-irradiated mice. The findings showed that DPSE injected into mice before irradiation has no toxicity and could protect mice from lethal effects of total body irradiation. Given that this work is the first study on evaluation of the radioprotector effect of DPSE in gamma-irradiated mice, hence, the use of DPSE as a new radioprotector in the human needs further studies, particularly clinical trials.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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