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Radioprotective Effect of Ferula Asafoetida Oleo-Gum Resin on Ionizing Radiation–Induced Oxidative Stress and Tissue Damage in Rats

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ABSTRACT

Introduction: Protective agents against harmful radiation have been studied for decades. Antioxidants can protect normal tissues by scavenging free radicals generated during irradiation. Ferula asafoetida (AS), a medicinal plant with antioxidant activity, was evaluated in this study for its protective effect against radiation-induced small intestinal injury in rats.

Material and Methods: Thirty-five Wistar rats were randomly divided into five groups (n=7): control, irradiated (R), irradiated+AS (R+AS), irradiated+vitamin E (R+E), and irradiated+AS+E (R+AS+E). Treatments included AS (100 mg/kg) and/or vitamin E (20 mg/kg) daily for eight days. On day six, irradiated groups received 6 Gy X-rays (6 MV, Elekta, Stockholm, Sweden). On day eight, rats were euthanized and intestine and liver tissues collected. Histopathology, malondialdehyde (MDA), and glutathione (GSH) levels were analyzed.

Results: AS significantly reduced MDA and increased GSH levels in both intestine and liver. Vitamin E showed weaker effects. The combination of AS and vitamin E did not consistently enhance AS activity. Histological analysis revealed that AS reduced inflammation and atrophy, while vitamin E alone or combined with AS lowered inflammation and epithelial erosion. Neither treatment increased mucous cell counts.

Conclusion: AS exerted notable antioxidant and radioprotective effects against intestinal damage in rats, indicating its potential as a natural agent for mitigating radiation-induced injury.

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Introduction

It is now well known that radiation can harm living organisms. This is why it's important to protect humans from radiation exposure in various fields like medical diagnosis and therapy, industry, energy production, and situations like air and space travel, nuclear accidents, and nuclear terrorism. Using lead shielding and other physical barriers can be difficult in these scenarios, so using drugs could be a good way to protect people from the harmful effects of radiation [1].

Scientists have been researching for many years to develop drugs that can protect against radiation damage. One of the main ways these drugs work is by scavenging free radicals, which are produced by ionizing radiation. Many natural antioxidants can help get rid of these free radicals and reduce the damage caused by oxidative stress. Therefore, finding natural substances that work in this way is very important [2].

According to Zakaryaee and Hosseinimehr (2013), antioxidants are compounds capable of neutralizing free radicals, thereby preventing tissue and organ damage. Both exogenous antioxidants, such as vitamins C and E and flavonoids, and endogenous systems—including glutathione, thioredoxin, and enzymes such as superoxide dismutase, glutathione peroxidase, and catalase—play critical roles in cellular defense against oxidative stress induced by radiation. Exogenous antioxidants or substances that stimulate endogenous antioxidant activity have also demonstrated radioprotective effects. Foods and dietary supplements rich in antioxidants are considered effective strategies for scavenging free radicals and minimizing their detrimental impact on

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healthy tissues. Moreover, recent studies have highlighted several plant-derived compounds as potential radioprotectors. These natural agents exhibit free radical scavenging activity, inhibit lipid peroxidation, and enhance reduced glutathione levels within tissues, supporting their protective role against radiation-induced cellular damage [3].

Ferula asafetida (AS) is a plant native to the central region of Iran. The stems and roots of AS are used in clinical applications, such as treating digestive system and spasm disorders. Pharmacological and biological studies on Ferula asafetida have revealed various medicinal properties, including antioxidant, antileishmanial, anticonvulsant, anti-diabetic, antispasmodic, hypotensive, and antinociceptive effects [4]. Additionally, Ferula asafetida has shown cancer-preventive and anti-mutagenic properties [5]. Research has suggested that the radical scavenging activities of AS are primarily due to its redox properties, which help neutralize free radicals, quench singlet and triplet oxygen, and decompose peroxides.

Studies analyzing the total phenolic content and total antioxidant capacity of certain medicinal plants have indicated a significant linear correlation between the total phenol content and antioxidant capacity. Furthermore, the use of flavonoids, a group of plant polyphenols with strong antioxidant properties and radical scavenging abilities, as regulators of redox-sensitive signaling pathways, is of particular interest. Previous studies have demonstrated that methanol extracts and essential oils from some Ferula species exhibit moderate antioxidant activity and radical scavenging capabilities. These radical scavenging effects are likely attributed, at least in part, to the presence of phenols, flavonoids, and sesquiterpenes in the extracts. Therefore, the varying antioxidant activities observed may be linked to the different phenolic and flavonoid contents in the essential oils obtained from various collections [6].

Considering the antioxidant properties of AS, it was expected that AS has radiation protection properties. Therefore, the present study was designed to investigate the radio-protective activity of AS. To this aim, following treatment with AS, the pathological damage induced by radiation in the rat's small intestine as well as Malondialdehyde (MDA) level and the total thiol content in the liver and small intestine of the rats were measured.

Based on the radiation studies conducted on rats [7, 8], we concluded that whole body radiation with 6 Gy X-rays can cause mucosal damage in the small intestine. For this reason, we exposed the mice to whole body irradiation of 6 Gy X-rays to cause visible damage in their intestines.

Materials and Methods

Preparation of Ferula asafoetida extract (AS extract):

AS gum was purchased from the Faculty of Pharmacy of Mashhad University of Medical Sciences. 220 grams of gum was ground into powder in the laboratory of the Faculty of Traditional Medicine of Mashhad University of Medical Sciences and soaked with 5000 cc of ethyl acetate at laboratory temperature for 48 hours. Then, for 48 hours, the container containing AS gum powder and ethyl acetate was placed at room temperature. During this time, the mixture was stirred intermittently. Afterward, the solution was completely filtered using filter paper. In order to prepare the dry extract, the obtained solution was placed in a fan oven at a temperature of 40 degrees for 72 hours. Finally, an amount of 120 grams of AS extract was obtained, which was kept in the freezer during the entire duration of this research.

Animal study:

Thirty five adult male Wistar rats, weighing between 200-250 grams, were purchased from the Laboratory Animal Research Center of Mashhad Medical School. Before starting the study, the animals were kept in the laboratory animal research center for one week to get used to the environmental conditions. Then the mice were randomly selected into five groups of 7 as follows.

1) The control group did not receive AS extract or radiation. This group was given corn oil by gavage (0.1cc per 10g weight of Rat body weight) once a day for 8 days.
2) The radiation group (R) received corn oil (0.1 cc per 10 g of body weight) daily by gavage for 8 days. On day six, irradiation was performed at the Radiotherapy Department of Imam Reza Hospital, Mashhad, using a linear accelerator (Compact Elekta, Stockholm, Sweden). Mice were fixed in custom cylinders, with three phantom slabs placed beneath their bodies. The isocenter was positioned at the center of each mouse, with the vertical laser aligned at midline. Irradiation was delivered at a dose rate of 200 cGy/min, at a source-to-surface distance (SSD) of 965 mm, in a 20×20 cm^2 field, so that the center of the mice received a total dose of 6 Gy. The gantry angle was set at 0°, directing the beam vertically from above.
3) The AS+R group received AS extract (100 mg /kg) once a day for 8 days and 6 Gy whole body 6 MV X-ray irradiation on the sixth day.
4) The E+R group received vitamin E (20 mg/kg) once a day by gavage for 8 days, and 6 Gy whole body 6 MV X-ray irradiation on the sixth day.
5) The AS+E+R group received AS extract (100 mg/kg) and vitamin E (20 mg/kg) once a day for 8 days and 6 Gy whole body 6 MV X-ray irradiation on the sixth day.

Within 48 hours after radiation, the rats were euthanized and their liver and intestine tissues were extracted. A part of the liver and small intestine tissues were separated for biochemical investigations (MDA and total glutathione content measurement) and the remaining were used for histopathological assessment by two pathologists.

Lipid peroxidation occurs as a result of cell damage in plants and animals and is used as a marker to measure

the level of oxidative stress in cells and tissues. Malondialdehyde is measured by the Thiobarbituric Acid Reactive Substances (TBARS) method, which is used in human, animal and plant samples. To measure the amount of malondialdehyde, 200 microliters of the prepared sample was mixed with 800 microliters of the reagent solution and placed in closed tubes for 45 minutes in a Bain-Marie at a temperature of 95 degrees Celsius. Finally, the desired sample was centrifuged and the absorbance of the supernatant of the centrifuged solution was read by a microplate reader (Epoch Biotek USA). In short, malondialdehyde reacts with thiobarbituric acid (TBA) at high temperature and produces a pink product that is measured by colorimetric method.

To measure the amount of glutathione, 100 mg of the homogenized sample was centrifuged at 9000 rpm for 15 minutes and an equal amount of Dithiobis-(2-nitrobenzoic acid)-5,5′(DTNB) and Glutathione Reductase (GR) Enzyme reagents were added to the samples transferred to the well. Then, the optical absorbance of the plates was read by a microplate reader (Epoch Biotek USA).

The histopathological test included investigating the amount of atrophy, reduction in the number of mucus-secreting cells, erosion and emptying of the epithelium, and cell inflammation. The histological scores were determined from 1 (normal) to 4 (severe damage) in four grades.

Statistical analysis

Values are reported as mean+ Standard Error (SE). Statistical analysis of data was carried out by computer using SPSS 16. The normality of the biochemical data distribution was checked using the Kolmogorov-Smirnov test. One-way ANOVA analysis was used to compare biochemical data in five groups, and Tukey's method was used to compare pairs of the groups. Mann-Whitney U test was used to compare the data of histopathology assessment between the groups.

Results

Figures 1a and 1b present MDA levels in the intestine and liver, respectively. Intestinal MDA was significantly decreased in the AS+R, E+R, and AS+E+R groups compared to the radiation group ($p < 0.05$). The reductions in AS+R and AS+E+R were greater than that in E+R ($p < 0.05$), indicating a stronger effect of AS alone or combined with Vitamin E. However, no significant difference was observed between AS+R and AS+E+R ($p > 0.05$), suggesting that the addition of Vitamin E did not further enhance the effect of AS. The combination effect of AS and Vitamin E corresponded approximately to the sum of their individual effects, without a significant synergistic interaction. Similar trends were observed in liver MDA levels (Figure 1b).

According to Figure 1b, the reduction of liver MDA in AS+R group was more than E+R and AS+E+R groups, but no significant difference was seen. Figures 2a and 2b show the amount of glutathione in the intestine and liver of the rats, respectively. As can be seen in Figure 2a, the increase in the level of intestinal Glutathione (GSH) in the AS+R group and the AS+E+R group was significant compared to the radiation group, but no significant difference was observed between E+R group and the radiation group.

As shown in Figure 2b, the decrease of GSH in E+R group was also significant compared to the AS+R group, but the partial decrease in the AS+E+R group was not significant compared to AS+R and E+R groups, meaning that the extract alone was able to neutralize the effect of radiation.

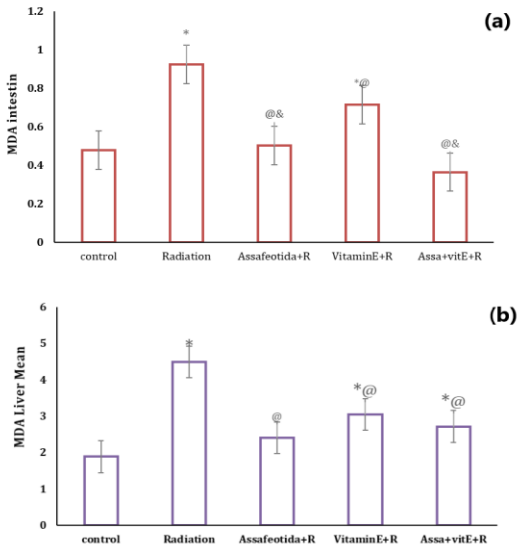

Figure 1. The average level of malondialdehyde (MDA) in the small intestine (a) and liver (b) of the rats. The * symbol on the top of the columns indicates a significant difference compared to the control group and the @ symbol indicates a significant difference compared to the R group. The & sign indicates a significant difference compared to the E+R group.

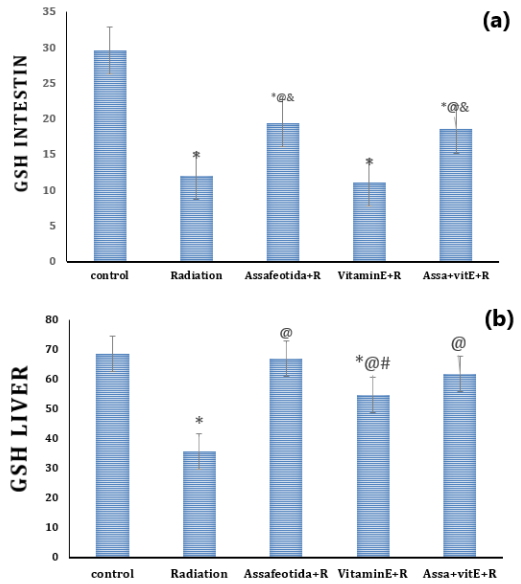

Figure 2. The average level of Glutathione (GSH) in the small intestinal (2a) and liver (2b) of the rats. The * symbol on the top of the columns indicates a significant difference compared to the control group and the @ symbol indicates a significant difference compared to the R group. The # and & signs indicate a significant difference compared to the AS+R group and E+R group respectively.

Figures 3 to 6 show the pathological damage to the intestine. To summarize the results related to the pathological damage induced in intestine, numbers 1 to 4 were assigned to different degrees of damage (normal= 1, mild damage= 2, moderate damage= 3, and severe damage= 4). Then, for each injury (in individual groups), the rats were sorted based on the assigned numbers (from low to high numbers) and the median was obtained numerically. Then, the medians of the 4 types of studied injuries were added together (Figure 7). As illustrated in Figure 7, the cumulated median decreased form 11 for group R to 5 for the control group.

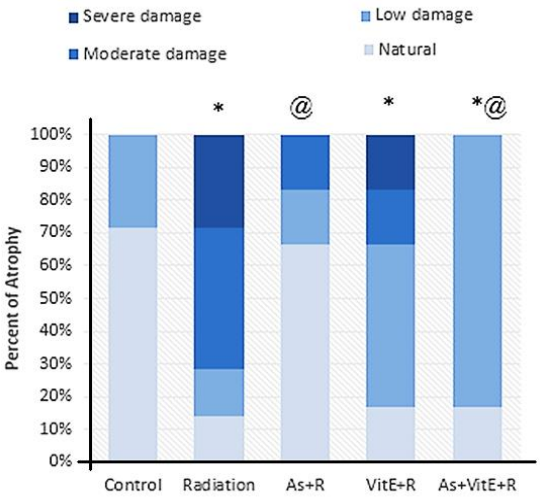

Figure 3. Percentage of the rats with various degrees of atrophy in the groups.
The * sign at the top of the columns indicates a significant difference compared to the control group and the @ sign indicates a significant difference compared to the R group.

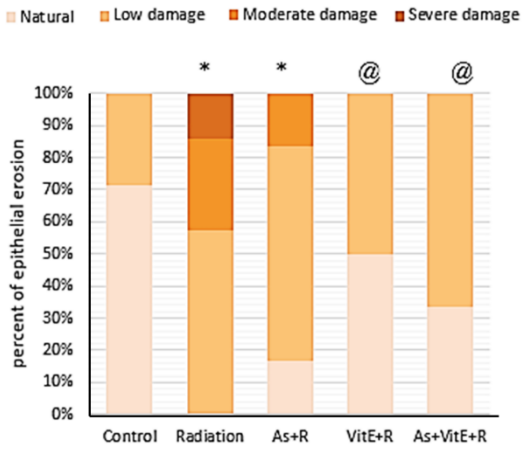

Figure 4. Percentage of the rats with various degrees of epithelial erosion in the groups.
The * sign at the top of the columns indicates a significant difference compared to the control group and the @ sign indicates a significant difference compared to the R group.

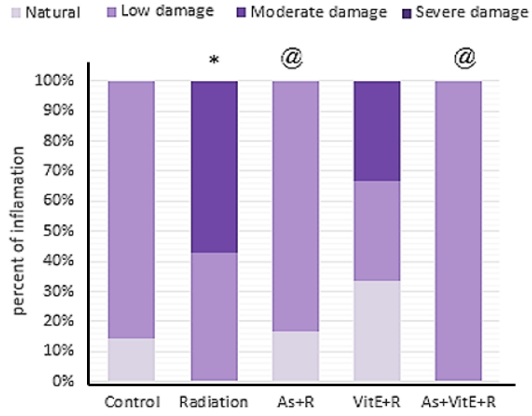

Figure 5. Percentage of the rats with various degrees of small intestinal inflammation in the groups.
The * sign at the top of the columns indicates a significant difference compared to the control group and the @ sign indicates a significant difference compared to the R group.

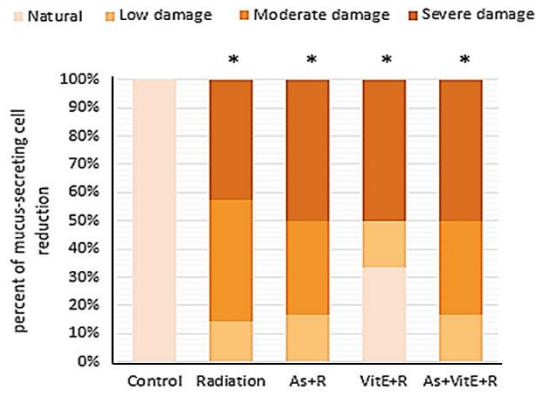

Figure 6. Percentage of the rats with various degrees of mucus-secreting cell reduction in the groups

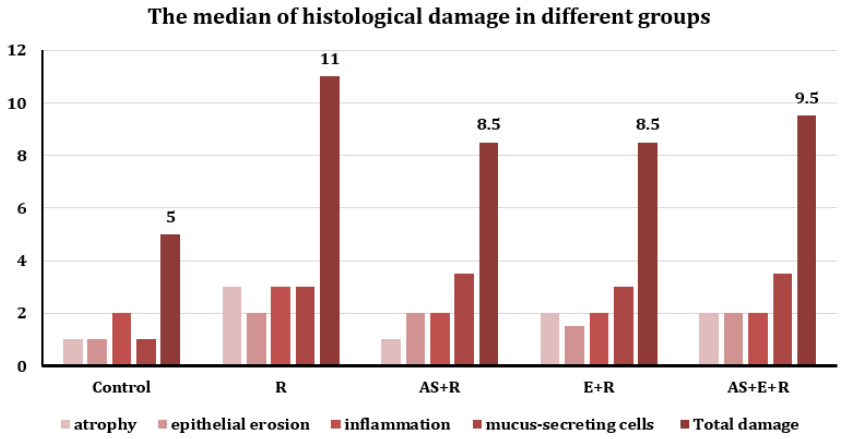

Figure 7. The median of different histological damage and cumulative median in individual groups

Discussion

The results showed that the MDA level in the groups that received AS (AS+R and AS+E+R), compared to the R group, decreased significantly. To explain of the reason, the results of a study carried out by Dehghan [9] can be referred. Their study showed that AS extract reduces lipid peroxidation and increases the activity of antioxidant enzymes. Furthermore, in accordance with our results, Esmaili [10] observed the improvement of blood liver parameters (Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST)) in rats that received AS extract through gavage for 1 month. In the present study, we also observed a significant difference in the GSH level of the intestine and the liver in the groups that received AS extract (groups AS+R and AS+E+R) compared to the R group.

The results also demonstrated that vitamin E with a dose of 20 mg/kg was able to significantly reduce the amount of MDA in the tissues of the small intestine and liver of the rats (there was a significant difference between groups E+R and R). This result is in agreement with the study of Mehrabadi and his colleagues [11]. They also observed that vitamin E could play an effective role in reducing the oxidative stress caused by the injection of Alzheimer's factor (beta-amyloid) in the hippocampus of the tested mice by reducing the MDA index and increasing the Superoxide Dismutase (SOD) index. Our results, however, indicate that the capability of vitamin E in reducing the oxidative stress induced by radiation is less than AS. Because, although the level of MDA in group E+R was lower than in group R, it could not decrease the amount of MDA to the level observed in the control group ($P < 0.05$ between groups E+R and control), while the level of MDA in the AS+R group was identical to its level in the control group ($P > 0.05$ between groups AS+R and R).

The combination of AS+R and vitamin E+R did not cause a greater decrease in MDA induced by radiation ($P > 0.05$ between groups AS+R and AS+E+R). In the intestine, the reason may be the fact that AS+R alone decreased the MDA level to the amount observed in the control group ($P <0.001$ between groups AS+R and control). However, in the liver, although there was not a statistically significant difference between the AS+R and AS+E+R groups, the numerical level of MDA was higher in group AS+E+R, so that the MDA level in group AS+E+R did not decrease to the level observed in the control group ($P < 0.05$ between groups AS+E+R and control). The results related to GSH showed the same trend. There was no significant difference in the amount of GSH in the intestine and liver of the groups AS+R and AS+E+R ($P < 0.05$ between groups). Regarding the liver, the level of GSH in the two groups reached the level observed in the control group. Therefore, more reduction in AS+E+R was not expected. However, regarding GSH level, we expected a higher amount of GSH in group AS+E+R compared to group AS+R. Because the level of intestinal GSH was significantly lower in the AS+R group than in the control. Nevertheless, we observed the same amount of GSH level in the AS+R and AS+E+R groups, which indicates vitamin E could not raise the positive impact of AS on the reduction of MDA level in the liver and the increase of GSH level in the intestine. While the vitamin slightly reduced the beneficial effect of the extract, no significant relationship was observed. In confirmation of our results, there are studies such as Deniz et al.'s study [12], which stated that AS can effectively increase liver glutathione levels. Also, Torabi et al.'s study [13], showed that AS can significantly improve the reduction of liver GSH levels in colon carcinogenesis. The pathological examinations showed that, although the two groups of AS+R and AS+E+R had no statistical difference in the rate of atrophy, and both had less atrophy than the R group, the reduction in the AS+R group was more than in the AS+E+R group. This observation indicates vitamin E decreased the radio-protective properties of AS in the case of atrophy. The lack of statistical difference between groups E+R and R confirms this claim. The opposite was correct for epithelial erosion. Vitamin E+R alone or in combination with AS+R could reduce epithelial erosion ($P < 0.05$ compared to group R, $P > 0.05$ compared to the control group), but there was no significant difference between groups AS+R and R while the AS+R group was significantly different from the control group. In relation

to the other two indicators, the effects of AS+R and vitamin E+R alone or in combination with each other were relatively similar. None of them had an effect on the increase of mucus-secreting cells ($P > 0.05$ compared to group R), but all three had a similar but not complete effect on reducing inflammation.

It indicates that AS and vitamin E had an identical radio-protective impact on the intestinal pathological injuries induced by radiation, but their combinatory impact was lower that their individual effects

Conclusion

The purpose of this research was to investigate the radio-protective effects of AS extract on the liver and small intestine tissue of the mice exposed to whole body irradiation (6 Gy). The results showed that AS extract effectively prevents oxidative stress caused by radiation, its effect is more than vitamin E, but its combination with vitamin E is not beneficial. It was also observed that the effect of AS extract and vitamin E in preventing radiation damage to the intestinal tissue is similar, but their combination reduces their efficiency. Therefore, it is suggested to use these two protective substances alone.

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