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Original Article

Effects of Arbutin on Radiation-Induced Micronuclei in Mice Bone Marrow Cells and Its Definite Dose Reduction Factor

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What's Known

- Previous studies have demonstrated that the Pyrus boissieriana Buhse leaves extract has antioxidant activity in normal rats. It protects skin against sunburn and is used as a whiting agent and also a protective agent against extreme UV radiation in pharmacological formulations.

What's New

- Arbutin effectively mitigated cytotoxic and clastogenic effects of gamma radiation, increased cell multiplication ratio to the normal level observed in the control group,and reduced the frequency of micronuclei by almost 2.22 times.
- This study introduces arbutin for the first time as a radio protector.

Abstract

Background: Interactions of free radicals from ionizing radiation with DNA can induce DNA damage and lead to mutagenesis and carsinogenesis. With respect to radiation damage to human, it is important to protect humans from side effects induced by ionizing radiation. In the present study,the effects of arbutin were investigated by using the micronucleus test for anti-clastogenic activity, to calculate the ratio of polychromatic erythrocyte to polychromatic erythrocyte plus normochromatic erythrocyte (PCE/PCE+NCE) in order to show cell proliferation activity.

Methods: Arbutin (50, 100, and 200 mg/kg) was intraperitoneally (ip)administered to NMRI mice two hours before gamma radiation at 2 and 4 gray (Gy). The frequency of micronuclei in 1000 PCEs (MnPCEs) and the ratio of PCE/PCE+NCE were calculated for each sample. Data were statistically evaluated using one-way ANOVA,Tukey HSD test, and t-test.

Results: The findings indicated that gamma radiation at 2 and 4 Gy extremely increased the frequencies of MnPCE ($P<0.001$) while reducing PCE/PCE+NCE ($P<0.001$) compared to the control group. All three doses of arbutin before irradiation significantly reduced the frequencies of MnPCEs and increased the ratio of PCE/PCE+NCE in mice bone marrow compared to the non-drug-treated irradiated control ($P<0.001$). All three doses of arbutin had no toxicity effect on bone marrow cells. The calculated dose reduction factor (DRF) showed DRF=1.93 for 2Gy and DRF=2.22 for 4 Gy.

Conclusion: Our results demonstrated that arbutin gives significant protection to rat bone against the clastogenic and cytotoxic effects of gamma irradiation.

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Introduction

Biologicalresponse oftissue toionizing radiationdepends onthephysical characteristic (type, energy, and dose rate) of ionizing radiation.[1] Whentissues are exposed to ionizing radiation, environmental conditions such as the existence of radio sensitizers and radio protectors could considerably change the amount of tissue damage. In a study, Alsbeih et al. showed

that the presence of oxygen as a radio sensitizer could significantly increase biological damage to ionizing radiation.[1] Mn products from whole chromosomes or centric chromosome fragments did not participate in cell division during anaphase. Mn formation could be considered as an appropriate biomarker of exposure to clastogenic and aneugenic hazard.[2] Studies revealed that, in the case of acute radiation exposure in human, Mn is a suitable biomarker for biological dosimetry. Mn frequency is affected by radiation dose.[3,4] Fenech showed an increased Mn frequency in human lymphocytes after exposure to 50-500 Msv range of ionizing radiation.[5] Mn assay had been utilized to evaluate the biological damage in populations living in areas with high levels of radioactivity[6] and in people who are occupationally exposed to ionization radiation.[7]

Arbutin is a glycosylated hydroquinone found in large amounts in many plants, including those from the families of rosaceous, ericaceous, etc.[8] The chemical formula for arbutin is $C_{12}H_{16}O_7$. Its molecular weight is 272.25 g/mol and it absorbs maximum UV light at 286 mm. It becomes unstable under heat and may decompose in different forms.[9] Peduncles, leaves, and bark of Pyrus plants contains certain amounts of arbutin. Arbutin content in the leaves of some Pyrusplants varies slightly from flower initiation to the time when leaves fall.[9] The best time for obtaining greater amounts of arbutin from young and green leaves of the Pyrus plants is spring.[10] In this study, we investigated the effects of arbutin administration in modulating the genetic damage induced by gamma irradiationin the mouse erythropoietin system by determining the frequency of micronuclei in immature erythrocytes of bone marrow.

Materials and Methods

Animal

Male NMRI mice (6 to 7 weeks old, 25±5 gr, number of groups=10, number of mice in each group=5, total mice=50) were used in this study. They were given unlimited fresh water and standard food. The mice were kept at standard ambient conditions, including appropriate temperature(22±1ºC), humidity, and light regime (12:12 hour light-dark cycle). Pure (98%) arbutin powder (Sigma) (50, 100, and 200 mg/kg) were dissolved in distilled water and injected into mice intraperitoneally, 2-h before irradiation. (figure 1)

Irradiation

Whole-body irradiation performed with a linear particle accelerator (LINAC) source (Elekta).

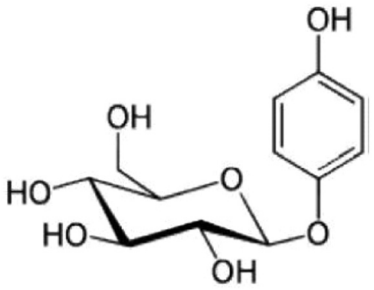

Figure 1: Shows chemical structure of arbutin.

Mice were placed in a ventilated plexiglas cage and irradiated in groups of five mice simultaneously. The source-to-skin distance of 100 cm with a dose rate of 200 cGy/min at room temperature (23±2ºC) was used in all experiments. Two doses of 2 Gy and 4 Gy were applied in this study.

Micronucleus Assay

Bone marrow obtained from the mice was tested using Schmidt's method for micronucleus test.[11,12] The control group tolerated all the stress except for the treatment. The radiation groups received 2 and 4 Gy gamma radiations and other groups received 50, 100, 200 mg/kg arbutin (ip), 2 hours before irradiation. 24 hours after irradiation, the mice were slaughtered using cervical dislocation. For each mouse, the marrow of both hip bones was collected in a test tube. The test tubes containing bone marrow and FCS were centrifuged at 1000 rpm for 7 minutes. Then, the solution over the deposited substances in each tube was discharged and about 100 µl of the serum remained in the tube. Next, 20 µl of the solution was placed on a slide, previously prepared for this purpose, and the other slide was used to uniformly spread the solution drop. The second slide was placed on the drop at 45° in order to spread the drop widthwise and the drop was then uniformly spread by rapid movements of the slide to the other side. The slides were kept for 24 hours at room temperature in the laboratory and then fixated using methanol before staining.

The samples were stained with May-Grunewald-Gyms as described by Schmidt.[12] May-Grunewald stain differentiates between PCE and NCE. Although Gyms stain is effective in this distinction, but its major role is in staining micronuclei. To achieve optimum staining conditions, different conditions were examined to obtain the following specifications:

5 min: Thick May-Grunewald stain

3 min: Diluted May-Grunewald stain (1:1 stain-to-distilled water ratio) cleansing by distilled water for two times

20 min: Gyms stain 5% final cleansing using distilled water.

The slides were dried at room temperature after staining. In a proper staining, nuclei of the nucleated cells become dark blue. NCEs become yellow-orange and PCEs become blue-violet. This leads to a clear distinction of the two types of cells. Micronuclei in PCEs and NCEs also become dark blue after staining.

Micronucleus test was used to assess cytogenetic effects of gamma radiation and the radio protective effects of arbutin. Once the slides were prepared, for each sample 1,000 PCEs and NCEs were counted along with nucleated PCEs and NCEs. In addition, PCE/PCE+NCE ratio was calculated for each mouse.

Microscopic and Statistical Analysis

The cells were counted using immersion oil and an Y100 Nikon microscope with ×100 objective lens. A total of 1000PCEs was scored for the presence of micronuclei for each sample. In order to study the cytotoxic effects of gamma rays on the proliferation of the bone marrow cells, the ratio of PCEs/PCEs+NCEs was calculated. The ratio is usually about 0.5 for the mice in the control group. Smaller ratios indicate cytotoxicity in the mice treated with radiation or a chemical. Greater ratios, on the other hand, shows an increase in cell multiplication as a result of the agent administered to the mice.

Statistical analysis using one-way ANOVA,Tukey HSD test and t-test were used to show the significance of any intergroup differences in the number of micronucleated PCEs and the ratio of PCEs/PCEs+NCEs.

Results

The results are shown in table 1. Statistical analysis showed that the groups that had received maximum doses of arbutin (200 mg/kg), displayed no significant differences in MnPCEs and cell proliferation ratio in comparison with the control group (P=0.5).

Therefore, lower doses of arbutin have not any clastogenic or cytotoxic effects. As shown in table 1, the gamma radiation 2 and 4 Gy caused a significant increase in the frequency of MnPCEs and decrease PCE/PCE+NCE in comparison with the control group (P<0.001).

The frequency of MnPCE found in the arbutin treated groups was significantly lower than the treated group with radiation alone (P<0.001).

In arbutin plus 2Gy groups, there were no significant difference in MnPCE and MnNCE between 50, 100 and 200mg/kg (P=0.5). In addition, no significant difference was found in PCE/PCE+NCE between the 100 mg/kg and 200 mg/kg groups (P=0.5). However, PCE/PCE+NCE in the group that received 50 mg/kg arbutin was significantly different from the corresponding ratio in the groups that were given 100 and 200 mg/kg arbutin (P<0.01).

In arbutin plus 4Gy groups, there was no significant difference in the frequency of MnPCEs and the ratio of PCE/PCE+NCE between 50, 100 and 200 mg/kg (P=0.5), thus the dose of 50 mg/kg could be considered as an optimal dose.

Table 2 Shows the dose reduction factor (DRF), the ratio of MnPCE in the treatment groups to the MnPCE in the radiation-only groups, for different doses of arbutin at 2 and 4 Gy gamma radiations.

$$DRF=\frac{\text{the amount of injury with radio protector in spesific radiation dose}}{\text{the amount of injury without radio protector in specific radiation dose}}$$

Table 1: Average frequencies of MnPCE and MnNCE and PCE/PCE+NCE (mean±SE) in the mice of the control group, 2 and 4 Gy radiation groups, and groups that received radiation and arbutin

Group	Mean±SE			P value		
	MnPCE/1000 PCE	MnNCE/1000 NCE	PCE/PCE+NCE	MnPCE	MnNCE	PCE/PCE+NCE
Control	5.00±1.01*	5.60±0.57	0.44±0.01			
Arbutin (200 mg/Kg)	5.30±0.57	5.60±0.57	0.45±0.01	0.99***	0.99	1.00
Gamma (2 Gy)	97.33±5.39	9.70±1.51	0.31±0.31	<0.001	0.00	<0.001
Gamma (4 Gy)	116.43±0.40	13.56±3.38	0.21±0.01	<0.001	0.00	<0.001
Arb (50mg/Kg)**+2Gy	47.33±2.51	7.00±0.01	0.43±0.02	<0.001	0.00	1.0
Arb (100mg/Kg)+2Gy	50.00±1.51	7.60±0.57	0.41±0.01	<0.001	0.46	<0.01
Arb (200mg/Kg)+2Gy	51.53±3.11	6.30±0.57	0.41±0.01	<0.001	0.46	<0.01
Arb (50 mg/Kg)+4Gy	52.33±2.50	5.60±0.57	0.39±0.01	<0.001	0.88	<0.01
Arb (100mg/Kg)+4Gy	52.00±2.61	5.30±0.57	0.39±0.01	<0.001	0.88	<0.01
Arb (200mg/Kg)+4Gy	52.00±2.60	6.00±1.01	0.39±0.01	<0.001	0.88	<0.01

*Data are mean±SE of 5 mice in each group; **Arbutin dosage is in mg/Kg and Arb denotes arbutin; ***P value compared to the control group

Table 2: DRF for different dosages of arbutin

Dose	2Gy DRF	4Gy DRF
Arbutin (50)	2.05	2.22
Arbutin (100)	1.94	2.23
Arbutin (200)	1.8	2.23

Discussion

The cytokinesis-block micronucleus (CBMn) assay is a biological dose estimates for unknown radiological exposures that evaluate the human population micronuclei.[13] It is a cytogenetic method for assessing cytotoxic effects such as ionizing radiation, chemical materials in mammalian system as well.[14]

The data clearly indicate that the mean frequency of Mn in groups exposed to 2 and 4 Gy gamma radiations is remarkably higher than the control groups. Previous cytogenetic research done by Maffie et al.,[15] Thierens,[16] and Khosravifarsani et al.[17] confirmed our data. In addition, the 2Gy group is significantly different from the 4 Gy group regarding the frequency of MnPCE ($P<0.05$), the frequency of MnNCE, and PCE/PCE+NCE ($P<0.001$). The 4Gy group increase MnPCE and MnNCE while reducing PCE/PCE+NCE ($P<0.001$) compared to the 2Gy group.

Previous studies by Hosseinimehr and Nemati,[18] Shahidi and Mozdarani,[19] and Shokrzadeh et al.[20] confirmed our data. Arbutin has diverse functions, including being an anti-oxidant and a depigmenting agent.[21] In erythrocytes, arbutin shows long-lasting radical scavenging properties and protects a membrane lipid from oxidative stress in human skin fibroblasts.[22,23] It has antioxidant properties, protects skin against sunburn, is used as whitening agent, and for protection against extreme UV radiation in pharmacological formulations. Arbutin does not cause skin or eye irritation.[24] Previous study by Gholizade et al., demonstrated the Pyrus boissierianabuhse leaves extract has antioxidant activity serum, liver, kidney and pancreas in normal rats.[25] Previous study by Ismail Shahaboddin et al. demonstrated the Pyrus boissieriana buhse leaves extract with 66% arbutin has antioxidant, antihyperlipidemic and antihyperglycaemic activity.[26] A similar decrease in MnPCE induced by gamma irradiation has been described by other antioxidants such as amifostine, glutathione, and cimetidine.[27,28] Treatment with arbutin at doses of 50, 100, 200 mg/kg before exposure to 2 and 4 Gy radiations reduced the frequency of MnPCEby almost 2.22 fold. Among radioprotective agents, amifostine has been evaluated as a powerful radio protector, but this drug is only effective at high doses, which is close to the toxic level (i.e., two-thirds of the Ld50 value) and induce side effects.[27]In this study, we showed arbutin protected mice bone marrow at the relatively low dose of 50 mg/kg.

Arbutin is widely used as cosmetic preparation,but the role of arbutin in the gene expression of human malignant melanoma cells is rarely studied.The genotoxic effect of arbutin and its side effects in cancer progression and melanocytic tumorigenis is reported.[29]

The effects of arbutin on clastogenic and cytotoxic conditions caused by gamma ray injection of arbutin at 200 mg/kg (the maximum dosage studied here) in the absence of radiation, does not significantly change frequencies of MnPCE,MnNCE and PCE/PCE+NCE compared to the control group (table 1). This means that the maximum dosage of arbutin examined here, does not have any clastogenic and cytotoxic effect. This also applies to smaller doses, i.e., 50 and 100 mg/kg.

Administration of arbutin prior to gamma radiation resulted in extreme reduction in the frequency of micronuclei. At 2 and 4 Gy gamma radiations, arbutin given at 50, 100, 200 mg/kg reduced MnPCE frequencies from 97.55, 98.22, and 98.65 to 46.30, 50, and 51.53, respectively, for 2 Gy and for 4 Gy groups from 116.40, 114.53, and 117.20 to 52.3, 52, and 52, respectively. However, no significant difference was found between the three doses in terms of MnPCE reduction ($P=0.5$). The frequency of MnNCE was also decreased. Therefore, 50 mg/kg can be considered as a desirable dosage. In addition, injection of arbutin at 50, 100, 200 mg/kg prior to radiation increased PCE/PCE+NCE from 0.29, 0.32, 0.31 in the 2 Gy group to 0.43, 0.41, 0.41, respectively, and for 4 Gy from 0.21, 0.23, 0.22 to 0.39, 0.39, 0.39, respectively (table 1). Therefore, no significant difference was observed between the groups that received 100 and 200mg/kg of arbutin with 2 Gy, while the 50 mg/kg group showed significantly different results from the other two groups ($P<0.01$). Therefore, the desirable dosage for arbutin is 50 mg/kg.

However, no significant difference was observed between the three doses arbutinplus4 Gy in terms of PCE/PCE+NCE.

In summary, based on the discussion above, it can be concluded that arbutin at different doses had radio protective properties and mitigated clastogenic effects of gamma ray. At both radiation levels, arbutin reduced the frequency of micronuclei by almost 1.5 times. As seen in the results, at 4 Gy gamma radiation, like 2 Gy radiation, arbutin

reduced micronuclei count and can therefore show radio protective properties in case of more severe radiation-related complications that occur at higher doses. Smaller doses of arbutin reduced micronuclei count. Arbutin has effectively mitigated cytotoxic effects of gamma radiation and increased cell multiplication ratio at both radiation doses to the normal level observed in the control group. A good radio protector used in radiotherapy patients should have the following properties: (i) offer a good protection against acute and chronic radiation damage (DRF>1.5), (ii) have the ability to be rapidly absorbed and distributed throughout the body, (iii) have no significant toxicity or side effects in the body, (iv) be chemically stable, and (v) be widely available and cost-effective.[30] Arbutin shows a good radiation protection. One of the benefits of this study is to introduce arbutin for the first time as radio protector, but since this study was conducted on mice, generalization to humans is difficult.

Conclusion

Our results demonstrate that arbutin gives significant protection to mice bone against the clastogenic effects of gamma irradiation. These features make them suitable for use as chemical radio protectors for different exposure situations,where there is a risk of damage to bone marrow.

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Conflict of Interest: None declared.

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