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Effect of Low-Dose Gamma Irradiation on Lipolytic Activity and Pepsinogen Level in Rat Salivary Glands and Blood Serum, and Their Correction with Bioflavonoids

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Abstract This study investigated the effects of 1 and 2 Gray (Gy) gamma irradiation on lipolytic activity and pepsinogen levels in the parotid salivary glands and blood serum of rats, as well as their correction with the bioflavonoids glabridin, pulicarin, and rutin. Rats were irradiated using a Terabalt-80 apparatus with a 60Co source at a dose rate of 0.86-0.85 Gy/min, with total absorbed doses of 1 and 2 Gy, and lipolytic activity and pepsinogen levels were examined on days 1, 3, 10, 20, 30, and 60 post-irradiation and after bioflavonoid administration. The results demonstrated that increasing radiation doses led to a decrease in lipolytic activity and pepsinogen levels, while bioflavonoids exerted a protective effect on these parameters. Rutin exhibited the highest efficacy, followed by pulicarin and glabridin. Bioflavonoids are recommended as promising natural agents for the correction of radiation-induced damage.

Keywords Gamma irradiation, Salivary glands, Lipolytic activity, Pepsinogen, Bioflavonoids, Rutin, Pulicarin, Glabridin, Radioprotector

1. Introduction

Ionizing radiation, particularly gamma irradiation, is a powerful stress factor that induces profound biochemical and functional alterations in living tissues. In radiotherapy for head and neck tumors, the salivary glands (parotid and submandibular) are among the most radiosensitive organs. Modern research indicates that gamma irradiation causes destruction and apoptosis of acinar cells in salivary glands [1], [2].

Salivary glands perform not only exocrine but also recretory and incretory functions. They have the ability to extract various metabolites and enzymes from the blood (recretion) and re-secrete them into saliva. The enzyme homeostasis in salivary gland secretions and blood — particularly the lipase (lipolytic activity) system involved in lipid metabolism — is highly sensitive to radiation [3].

According to the literature, the effect of gamma irradiation is dose-dependent. At low doses (0.5–2 Gy), short-term compensatory activation or mild functional changes in enzyme secretion are observed. At higher doses (4 Gy and above), severe hyposalivation (dryness), disruption of cell membrane permeability, and damage to recretory pathways occur [4]. Consequently, lipolytic activity in saliva decreases, while pathological increases in plasma lipolytic activity may occur due to enzyme leakage into the blood (hyperfermentemia).

The damaging effects of gamma radiation on tissues occur through two main mechanisms: direct (breakage of biomolecules and DNA strands) and indirect (formation of free radicals due to water radiolysis).

Contemporary studies confirm that the primary factor in salivary gland injury is nitro-oxidative stress. Radiation exposure leads to a sharp increase in reactive oxygen species (ROS) in cells, which promotes lipid peroxidation (LPO) in lysosomal and cell membranes. ROS oxidize sulfhydryl (SH) groups in the active centers of enzymes, including lipase, thereby reducing their catalytic activity, and disrupt mitochondrial ultrastructure, ATP synthesis, and calcium signaling pathways.

Currently, due to the high toxicity of synthetic radioprotectors in medicine and radiobiology, there is growing interest in bioflavonoids derived from natural sources. Bioflavonoids (e.g., quercetin, rutin, hesperidin, curcumin, and epigallocatechin -3-gallate) have low toxicity and can be used long-term for prophylactic purposes [5]. Literature analysis shows that the protective mechanisms of bioflavonoids manifest in various ways [6].

Flavonoids neutralize ROS and free radicals through their phenolic rings, thereby reducing LPO and malondialdehyde (MDA) levels. They enhance Nrf-2 (nuclear factor erythroid 2–related factor 2) signaling, which coordinates the activity of intracellular superoxide dismutase, catalase, and glutathione

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systems. They maintain the integrity of acinar cell membranes and excretory duct membranes in the salivary glands, ensuring normal recretion of lipase and protecting enzymatic homeostasis between blood and saliva. Flavonoids also suppress the synthesis of pro-inflammatory cytokines (TNF-α, IL-1β) and inflammatory mediators, and block caspase-3 activity, preventing cell death [7]. The dynamic changes in lipolytic activity in the parotid salivary gland and blood under 1 and 2 Gy gamma irradiation, and the effects of flavonoids on these parameters, have not yet been studied.

The aim of this work was to study the effect of low-dose gamma irradiation on lipolytic activity and pepsinogen levels in rat salivary gland homogenates and blood serum, and to evaluate their correction with bioflavonoids. Rats were irradiated using a Terabalt-80 apparatus with a 60Co source at a dose rate of 0.86–0.85 Gy/min, with total absorbed doses of 1 and 2 Gy, and lipolytic activity and pepsinogen levels were examined on days 1, 3, 10, 20, 30, and 60 post-irradiation and after bioflavonoid administration.

2. Materials and Methods

2.1. Animals and Experimental Design

A chronic experiment was conducted on rats. Adult male outbred white rats weighing 175–200 g were used. The animals were kept under standard vivarium conditions and fed a carbohydrate-protein mixed diet with free access to water. The rats were divided into five groups: I — control (healthy); II — irradiated with 1 or 2 Gy gamma radiation; III — irradiated (1 or 2 Gy) + glabridin; IV — irradiated (1 or 2 Gy) + pulicarin; V — irradiated (1 or 2 Gy) + rutin.

The control group received no treatment. Rats in groups II–V were irradiated locally (head and neck area) using a Terabalt-80 apparatus with a 60Co source. The irradiation field was 20×20 cm, focus distance 80 cm, dose rate 0.86–0.85 Gy/min, and absorbed doses were 1 and 2 Gy. Flavonoids (glabridin, pulicarin, rutin) were administered at 50 mg/kg via gastric tube once daily, starting 5 days before irradiation and continuing after irradiation. Irradiated rats were kept in standard cages and decapitated on days 1, 3, 10, 20, 30, and 60 post-irradiation.

2.2. Determination of Lipolytic Activity

Lipolytic activity was determined by the Tietz method, based on titration of free fatty acids released during tributyrin lipolysis with 0.05 N NaOH [8], [9]. Lipase activity was expressed in Tietz units, calculated as the difference in alkali consumption between test and control tubes. The assay procedure was as follows: 4 ml of emulsion (containing 1.5 ml TRIS-hydroxymethylaminomethane buffer, 1.0 ml 0.1% CaCl2, 2 ml sodium cholate mixture, 0.25 ml tributyrin, and 0.25 ml distilled water) was added to each test and control tube. Then, 1 ml of test material (salivary gland homogenate or blood serum) was added. The control tube received 3 ml of 96% ethanol immediately to stop the reaction. The test tube was incubated for 2 hours at 37 ℃ in a water bath, after which the reaction was stopped by adding 3 ml of 96% ethanol. After stopping the reaction, 3–4 drops of thymolphthalein were added, and titration with 0.05 N NaOH was performed until a stable blue color appeared. One unit of lipase activity was defined as the amount of alkali consumed to reach a faint pink endpoint.

2.3. Determination of Pepsinogen Levels

Pepsinogen levels in salivary gland homogenates were determined using a modified Hirschowitz tyrosine method [10], [11]. In our modification, the incubation time was extended to 24 hours, and dried plasma was used as the protein substrate instead of hemoglobin. For analysis, 0.5 ml of test material (salivary gland homogenate or blood plasma) was mixed with 2.5 ml of 2% plasma solution in 0.05 N HCl. The mixture was incubated at 37 ℃ for 24 hours. A parallel control tube containing 2.5 ml of 2% plasma solution in 0.05 N HCl was also incubated. After incubation, 0.5 ml of test material was added to the control tube. Then, 2.5 ml of 0.5 N trichloroacetic acid (TCA) was added to both tubes, and the mixture was filtered through thick filter paper. To 1 ml of filtrate, 2.0 ml of 0.5 N NaOH and 0.6 ml of Folin–Ciocalteu reagent (diluted 1:2 with water) were added. The resulting solution was colorimetried against water using a red filter for 3–20 minutes. The extinction of the control tube was subtracted from that of the test tube. The difference was converted to arbitrary activity units using a calibration curve prepared with tyrosine. One unit of pepsinogen activity was defined as the amount of enzyme that released 1 μg of tyrosine from protein substrate at 37 ℃ over 24 hours.

2.4. Statistical Analysis

Statistical processing of the obtained results and plotting of figures were performed using OriginPro 8.6 (Microsoft, USA) computer software. Experimental data were statistically processed according to the Student–Fisher method and subjected to correlation analysis. Differences between the values obtained from the control, experimental, and experimental + test substance groups were calculated using the t-test, where $P<0.05$ and $P<0.01$ indicate statistical significance.

3. Results and Discussion

Salivary gland secretions contain lipase primarily via the recretory pathway. We studied the effect of different doses of gamma irradiation on lipolytic activity in rat salivary glands and blood.

3.1. Lipolytic Activity in Salivary Glands

To evaluate the dose-dependent effect of ionizing radiation and the radioprotective potential of natural polyphenols, we examined the dynamics of lipolytic activity in the parotid salivary gland from day 1 to day 60 under 1 Gy and 2 Gy gamma irradiation (Figure 1 and Figure 2). The results showed that increasing radiation dose significantly

deepened enzyme suppression, and the protective effect of flavonoids was proportionally dose-dependent.

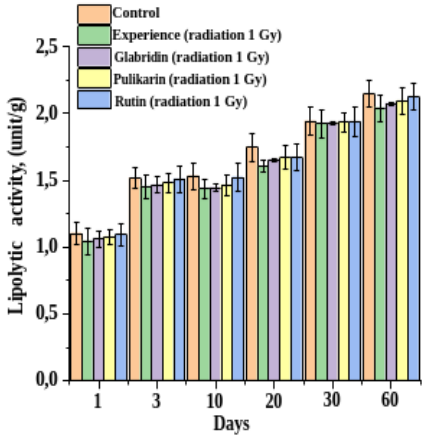

Figure 1. Effect of 1 Gy gamma irradiation on lipolytic activity (U/g) in the parotid salivary gland. (All cases: significance level P<0.05; n=6)

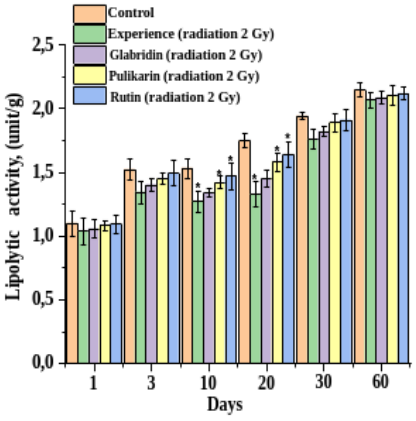

Figure 2. Effect of 2 Gy gamma irradiation on lipolytic activity (U/g) in the parotid salivary gland. (All cases: significance level P<0.05; n=6)

Lipolytic activity in salivary glands. In the control groups, lipolytic activity steadily increased from day 1 to day 60 due to ontogenetic development (from 1.103 to 2.147 and from 1.101 to 2.147). However, under gamma irradiation, a sharp dose-dependent decrease in enzyme activity was observed.

At 1 Gy, the lowest enzyme activity occurred on days 10 and 20, reaching 1.4367 ±0.068 and 1.6067 ±0.04, respectively (a decrease of approximately 6–8% compared to control). By days 30 and 60, the values approached the control level (2.0417 ±0.101 on day 60).

At 2 Gy, the extent of radiation damage increased sharply. Enzyme depression was not only deeper but also prolonged. The lowest value was recorded on day 10 (1.27 ±0.08, a 17.1% decrease compared to control). Notably, on day 20, activity remained deeply depressed (1.33 ±0.1), and only from day 30 onward was a slow recovery observed (1.76 ±0.12). This dose dependence is explained radiobiologically: at 1 Gy, partial cell damage and transient enzymatic inhibition occur; at 2 Gy, radiation destruction of parotid acinar cells and deep damage to the blood-saliva barrier (histohematic barrier) occur, leading to prolonged disruption of lipase recretion from blood into saliva.

The tested flavonoids (glabridin, pulicarin, rutin) demonstrated protective effects on the enzyme system at both irradiation doses, but their efficacy varied.

Rutin proved to be the most potent radioprotector at both 1 and 2 Gy. At 1 Gy, it maintained enzyme activity close to control levels throughout most time points (day 10: 1.522 ±0.102 vs. control 1.533 ±0.101). At 2 Gy, even under severe radiation suppression, rutin exhibited strong angioprotective and antioxidant properties, maintaining lipolytic activity at 1.47 ±0.102 and 1.64 ±0.1 on days 10 and 20, respectively — significantly higher than the irradiated control (1.27 and 1.33) (Figure 1 and Figure 2). Rutin's polyphenolic rings effectively bind free radicals and strengthen capillary walls, maintaining recretory filtration.

Pulicarin (group 4) ranked second in efficacy. At 1 Gy, its results were close to those of rutin (e.g., day 20: 1.67 ±0.09). At 2 Gy, it effectively preserved enzyme activity (day 10: 1.42 ±0.05; day 20: 1.58 ±0.07), indicating strong membrane-stabilizing effects even at high radiation doses.

Glabridin (group 3) showed the weakest protective effect. At 1 Gy, a modest protective effect was observed (day 20: 1.654 ±0.0102), but at 2 Gy, its protective potential lagged significantly behind the severity of radiation damage. For example, on days 10 and 20, enzyme activity (1.34 ±0.034 and 1.45 ±0.07) was only slightly higher than the irradiated group (1.27 and 1.33), but much lower than control and other flavonoid-treated groups.

Thus, gamma irradiation suppresses the recretory lipolytic activity of the parotid salivary gland in a dose-dependent manner: at 1 Gy, transient (intensive phase: 10–20 days), and at 2 Gy, deep and prolonged (intensive phase: 10–30 days) reduction. Regardless of dose, flavonoids exhibited clear radioprotective effects. The efficacy order remained: Rutin > Pulicarin > Glabridin.

3.2. Lipolytic Activity in Blood

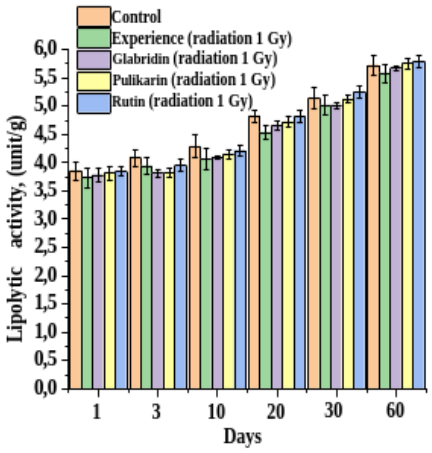

Figure 3. Effect of 1 Gy gamma irradiation on lipolytic activity (U/g) in blood. Ordinate — enzyme activity (U/g), abscissa — experimental days. (All cases: significance level P<0.05; n=6)

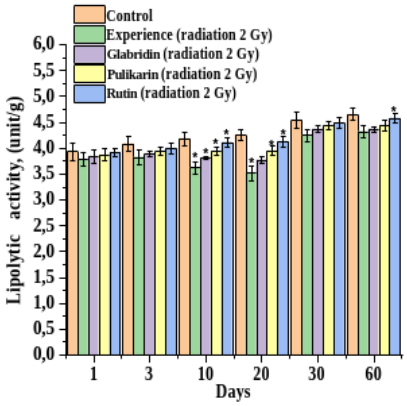

Figure 4. Effect of 2 Gy gamma irradiation on lipolytic activity (U/g) in blood. Ordinate — enzyme activity (U/g), abscissa — experimental days. (All cases: significance level P<0.05; n=6)

We simultaneously studied lipolytic activity in the blood of irradiated rats (Figure 3 and Figure 4). The results showed that low-dose gamma irradiation affects the secretory and incretory processes of the glands similarly. Lipolytic activity in salivary gland homogenates showed no significant changes after 1–2 Gy irradiation; however, at 2 Gy, blood lipolytic activity remained at control levels on days 3 and 10.

Ionizing gamma radiation sharply increases the generation of free radicals and reactive oxygen species in the body. This leads to lipid peroxidation of the acinar cell membranes of the pancreas and salivary glands and blocks the synthesis and secretion of lipolytic enzymes [12]. Oral administration of flavonoids 5 days before and after the experiment enabled effective scavenging of free radicals and protection of cell membranes from radiolysis products due to their phenolic hydroxyl groups. Among the studied compounds, rutin demonstrated itself as the most promising agent in protecting endogenous antioxidant systems and preventing amylase enzyme degradation owing to its polyphenolic structure and high antioxidant potential.

Correlation analysis between blood and parotid salivary gland lipolytic activity is shown in Table 1. The correlation coefficients were positive and high, ranging from 0.49 to 0.89, indicating a strong relationship between lipolytic activity in blood and salivary glands.

Table 1. Correlation coefficients (r) between blood and parotid salivary gland lipolytic activity (U/g) after different doses of gamma irradiation (n=6)

Experiment day	1 Gy	2 Gy
1 day, n=6	0.89	0.64
3 day, n=6	0.82	0.78
10 day, n=6	0.88	0.49
20 day, n=6	0.87	0.78
30 day, n=6	0.81	0.70
60 day, n=6	0.85	0.56

3.3. Pepsinogen Levels in Salivary Gland Homogenates

The effect of low-dose (1 and 2 Gy) gamma irradiation on pepsinogen content in rat salivary gland homogenates is shown in Figure 5 and Figure 6. A clear dose-dependent change was observed. At 1 Gy, pepsinogen levels in all salivary glands did not change significantly and remained at control levels (Figure 5). However, at 2 Gy, notable changes occurred.

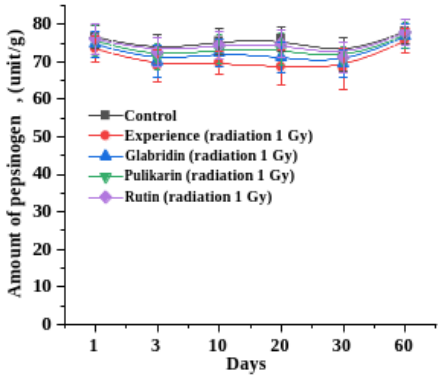

Figure 5. Effect of 1 Gy gamma irradiation on pepsinogen levels (U/g) in the parotid salivary gland. (All cases: significance level P<0.05; n=6)

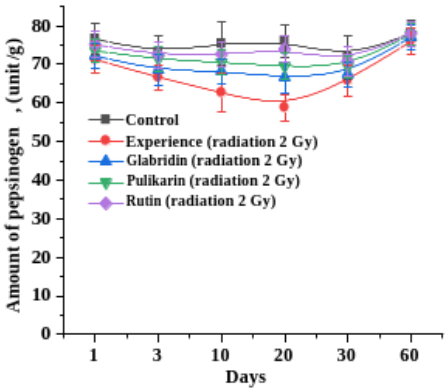

Figure 6. Effect of 2 Gy gamma irradiation on pepsinogen levels (U/g) in the parotid salivary gland. (All cases: significance level P<0.05; n=6)

Ionizing radiation negatively affects the secretory function of oral organs, particularly the salivary glands. Pepsinogen content in salivary gland homogenate is an important indicator of enzymatic activity and functional status.

At 2 Gy (group 2), pepsinogen levels in the parotid gland showed a decreasing trend compared to control. Although no sharp drop was observed in the early days, the lowest values were recorded on day 30 (75.3±2.06 vs. control 81.5±1.89), indicating progressive destructive changes in the parenchyma and gradual suppression of enzyme synthesis. By day 60 (79.25±1.61), partial recovery was observed (Figure 6).

The tested bioflavonoids showed varying degrees of protection against decreased enzymatic activity. Glabridin (group 3) prevented a sharp decline, maintaining levels at 77.5±3.1 on day 30 — slightly below control but higher than the irradiated group. Pulicarin (group 4) exhibited higher protective effects, maintaining stable pepsinogen levels throughout the experiment, with day 60 values (81.4±3.1) nearly returning to control levels (81.9±1.99). Rutin (group 5)

showed the strongest radioprotective properties. Even at the critical 20–30 day period, pepsinogen levels (80.5±3.7 and 80.5±4.1) did not differ significantly from controls (81.4±2.11 and 81.5±1.89). On day 60, values even exceeded control levels (82.3±3.5).

In conclusion, 2 Gy gamma irradiation causes delayed (up to day 30) reduction of pepsinogen in the parotid salivary gland. Among the tested substances, pulicarin proved to be a natural corrective agent with efficacy close to rutin in protecting the enzymatic system of the salivary gland from radiation damage.

3.4. Pepsinogen Levels in Blood

The dose-dependent effect of gamma irradiation on blood pepsinogen levels is shown in Figure 7 and Figure 8. At 1 Gy, blood pepsinogen levels did not change and remained at control levels.

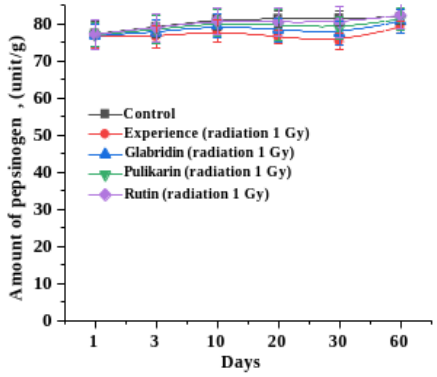

Figure 7. Effect of 1 Gy gamma irradiation on pepsinogen levels (U/g) in blood. (All cases: significance level P<0.05; n=6)

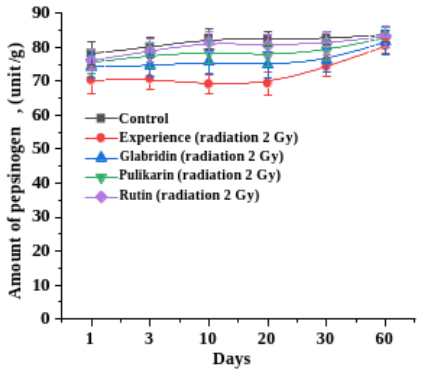

Figure 8. Effect of 2 Gy gamma irradiation on pepsinogen levels (U/g) in blood. (All cases: significance level P<0.05; n=6)

Ionizing radiation causes profound functional and structural disturbances in the gastrointestinal tract. Blood pepsinogen level is an important biochemical marker of gastric mucosal functional status. At 2 Gy (group 2), blood pepsinogen levels decreased significantly compared to controls. The lowest values were recorded on days 10 (69.07±2.65) and 20 (69.32 ±3.4), versus control values of 82.5±3.03 and 82.37±2.11, respectively. By day 60 (80.32±2.2), values approached control levels due to natural recovery (Figure 8).

The tested bioflavonoids demonstrated varying degrees of mitigation of radiation-induced adverse effects. Glabridin (group 3) partially protected gastric mucosa, maintaining pepsinogen levels around 74–75 throughout all time points, especially on days 10–20. Pulicarin (group 4) showed higher efficacy than glabridin, with pepsinogen levels actively recovering from day 3 and approaching control values (79.1±3.1) by day 30. Rutin (group 5) exhibited the highest radioprotective and corrective properties. In the rutin-treated group, pepsinogen levels reached near-control values (81.5± 3.0 vs. control 82.5±3.03) as early as day 10 and remained stable thereafter (Figure 8).

Thus, 2 Gy gamma irradiation suppresses pepsinogen synthesis in rats. Among the tested bioflavonoids, rutin and pulicarin showed the most effective restorative properties and can be considered promising natural agents for the treatment and prevention of post-irradiation gastrointestinal pathologies.

3.5. Correlation Between Blood and Salivary Gland Pepsinogen Levels

To determine the relationship between pepsinogen levels in blood and salivary glands, we calculated correlation coefficients (Table 2). The data show that with increasing radiation dose, the correlation between blood and parotid salivary gland pepsinogen levels becomes stronger. For example, at 1 Gy, correlation coefficients ranged from 0.34 to 0.75; at 2 Gy, from 0.52 to 0.74. These results indicate that as radiation dose increases, blood pepsinogen levels and its excretion via the parotid salivary gland decrease in the same direction.

Table 2. Correlation coefficients (r) between blood and parotid salivary gland pepsinogen levels (U/g) after different doses of gamma irradiation (n=6)

Experiment day	1 Gy	2 Gy
1 day, n=6	0.65	0.67
3 day, n=6	0.75	0.61
10 day, n=6	0.37	0.58
20 day, n=6	0.34	0.52
30 day, n=6	0.69	0.74
60 day, n=6	0.44	0.56

Gamma radiation dose-dependently suppresses the recretory lipolytic activity of the parotid salivary gland. At a dose of 1 Gy, a transient decrease in activity is observed (intensive period 10–20 days), whereas at 2 Gy, the reduction is profound and prolonged (intensive period 10–30 days). Flavonoids exhibit a pronounced radioprotective effect in protecting lipolytic activity regardless of the radiation dose of 1 or 2 Gy [13].

The obtained scientific results demonstrated that the radioprotective efficacy of these three bioflavonoids follows the order Rutin > Pulicarin > Glabridin. The differential activity of these compounds is directly related to their chemical structure, the number and position of hydroxyl groups in their composition, and their free radical scavenging potential.

As radioprophylactic agents, these natural phenolic compounds, especially pulicarin and rutin, hold high promise for ensuring the metabolic stability of salivary glands.

4. Conclusions

Gamma irradiation exerts a negative effect on the recretory function of rat salivary glands, lipolytic homeostasis in the blood–saliva system, and pepsinogen activity. The administration of natural bioflavonoids before or after irradiation demonstrates highly effective radioprotective effects by reducing oxidative stress, protecting membranes, and stabilizing enzyme activity. Among the tested compounds, the radioprotective efficacy follows the order Rutin > Pulicarin > Glabridin, which is directly related to their chemical structure, the number and position of hydroxyl groups, and their free radical scavenging potential. This substantiates the significant theoretical and practical potential of such research for medicine, and rutin and pulicarin in particular hold high promise as natural radioprotective agents for ensuring the metabolic stability of salivary glands.

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