

Simulation Of Radiation-Thermal Illness And A Way To Its Treatment

Timur R. Gaynutdinov¹, Airat M. Idrisov¹, Konstantin N. Vagin¹, Yagafar M. Kurbangaleev¹, Gulnaz Sh. Zakirova¹, Kamil T. Ishmukhametov¹, Farit H. Kalimullin¹, Rustam N. Nizamov¹, Vanentina A. Guryanova¹, Sergey Yu. Smolentsev^{2*}

¹Federal Center for Toxicological, Radiation and Biological Safety, Scientific town-2, Kazan city, 420075, Russia

²Mari State University, Lenin Square 1, Yoshkar-Ola city, 424000, Russia Smolentsev82@mail.ru

ABSTRACT.

Experimental modeling of acute radiation and burn disease was performed both separately and in combination. It was established that external total gamma-irradiation of white rats at the dose of 7.5 Gy causes radiation sickness of a heavy degree with characteristic clinical signs and high lethality of irradiated animals. The modeling of thermal shock by application of a heated to 190 °C metal plate with the holding exposition of 5 and 8 seconds was performed. It was found out, that the burn degree depends on the time of contact with the body surface: its application for 5 seconds caused a burn of III-A degree, 8 - second exposition - IIIB degree, which was proved by the terms of formation, rejection of the eschar and complete healing of thermal lesions. The aggravating effect of ionizing radiation on the course and outcome of combined radiation-thermal pathology was demonstrated. Treatment of studied pathology was carried out with purified turpentine which was injected in doses of 0.2; 0.4; 0.6 and 1.0 ml directly under burned skin area in first hours (1-24) after two-factor (radiation and thermal) exposure to organism. It was experimentally shown that an optimum therapeutic dose of purified turpentine is 0.4 ml under the condition of a single injection of the preparation under the wound surface, which was characterized by 83.3% survival rate and healing of burn wounds on the 36th day of the experiment.

Key words: radiation-thermal injury, therapy, purified turpentine.

INTRODUCTION.

The increasing use of ionising radiation sources in industry, science, health care, agriculture for power generation, defense, etc., the violation of the rules of operation of nuclear cycle facilities, transportation and storage of radioactive substances, can lead to disastrous consequences (Vaiserman et al. 2018). Radiation sickness is a pathological process that develops as a result of complex chemical and biochemical changes occurring in the body as a result of exposure to radiation. The final stage of radiation damage is characterized by visible functional and then morphological changes, arrest of cell division, chromosome breakage, cell death (Wang et al. 2019).

Radiation and thermal burns are among the pathological consequences of nuclear explosions and radiation accidents. While the former arise from the combined (simultaneous or sequential exposure of the body to external and local radiation), the latter result from the thermal effects of the occurred fires (Rios et al. 2020). A burn is tissue damage caused by local heat (thermal), chemical, electrical or radiation effects. Thermal burns are the most common type of burn in clinical practice (Friedstat et al. 2017). The severity of the injury depends on the temperature, duration of

exposure to the heat factor, the extent of the injury, and burn location. Flames, molten metal, pressurized steam, boiling oil, and hot gas cause particularly severe burns and are accompanied by various degrees of burns (Jeschke et al. 2020).

This is exemplified by the bombing of well-known Japanese cities and the events at the Chernobyl and Fukushima nuclear power plants when, in addition to ionizing radiation, people and animals were exposed to other damaging factors, such as light and blast waves, thermal factors, etc., causing combined radiation injuries, including thermal-radiation injuries (TRI), in the emergency situations created. The course of such pathological processes has its own peculiarities, so the treatment of burns received against the background of external gamma-irradiation is very relevant (Socol, 2015).

Preparations based on substances of microbial, phytogetic, zoogenic, and chemical origin are being intensively developed (Gaynutdinov et al. 2021). Research in recent years has shown that preparations of plant origin from the class of terpenoids have a stress-protective effect by inhibiting toxic radicals - products of lipid peroxidation - malondialdehyde, which play a key role in the development of radio-induced radiotoxemia and thermo-induced thermotoxemia (Proshkina et al. 2020).

The inclusion of phytopreparations into the potential radioprotective, thermal and bioprotective preparations is due to the fact that, firstly, they are low-toxic and easily available, secondly, they provide an increase in endogenous resistance background through the induction of synthesis of antiradical and anti-oxidative substances, providing stimulation of hematopoiesis, immunity, bone marrow cellularity, normalization of nucleic acids content, thirdly, they possess high anti-inflammatory and regenerative (wound healing) action, fourthly, they possess anti-radiation, sorption (decorporation) and anti-oncogenic action and, finally, they have an anti-burn action (Kuruba and Gollapalli, 2018). The bioprotective effect of phytopreparations (extracts, juices, powders) is attributed to the influence on the organism of not only one but many components of biologically active substances, which are sometimes in complementary and increasing interaction of biogenic compounds balanced by nature. One of the biologically active substances of plant origin from the class of terpenoids is turpentine oil - turpentine (Samarth et al. 2017). Based on the above, the aim of the research is to find an optimal model of combined radiation-thermal pathology and to develop ways to its treatment.

Materials and Methods. The experiments were carried out in several stages. The first stage of the work was carried out on white mongrel rats with an average weight of 180-200 g, divided into 6 groups, 5 animals in each: 1 - exposure to 7.5 Gy + burn, exposure 5 sec; 2 - exposure to 7.5 Gy + burn, exposure 8 sec; 3 - exposure to 7.5 Gy - control of exposure; 4 - burn, exposure 5 sec; 5 - burn, exposure 8 sec; 6 - biological control.

Simulation of acute radiation sickness was carried out using the "Puma" gamma unit with a ^{137}Cs source and an exposure dose rate of 2.26×10^{-5} A/kg at a dose of 7.5 Gy. Thermal burns were applied after exposure to ionising radiation. For that purpose, each animal was fixed by immobilizing each limb separately on a special machine. The thermal injury was inflicted by applying a brass heel (d=25 mm) heated to 190 °C to the cut skin area of the upper third of the thigh with an exposure time of 5 and 8 seconds ("Ricci" oven, activated carbon regenerator, model TO 10BTQS, oven size 450x330x350, weight 3 kg). For the burn injury, two brass heels were used (one was left in the oven at the time of the

other animal's burn injury). In terms of skin and underlying tissue damage, the burns were grade IIIA and IIIB, respectively.

Experienced animals were clinically monitored on a daily basis for behavioral reactions, feed intake, water consumption, visible mucous membranes, coat, and mobility.

In the second stage of work, the optimum therapeutic dose of purified turpentine was determined. For this purpose, the mongrel white rats of both sexes with an average live weight 200-220 g were selected. The rats were divided into experimental and control groups of 6 animals each according to the scheme: 1 - irradiation with a dose 7.5 Gy + burn of IIIB degree + treatment with purified turpentine with a dose 0.2 ml; 2 - irradiation with a dose 7.5 Gy + burn of IIIB degree + treatment with purified turpentine with a dose 0.4 ml; 3 - irradiation with a dose 7.5 Gy + burn of IIIB degree + treatment with purified turpentine with a dose 0.6 ml; 4 - 7.5 Gy irradiation + IIIB degree burn + purified turpentine treatment with a dose of 1.0 ml; 5 - 7.5 Gy irradiation + IIIB degree burn + treatment with 10% liniment of synthomycin - treatment control; 6 - 7.5 Gy irradiation + IIIB degree burn; 7 - IIIB degree burn without irradiation; 8 - irradiation control; 9 - biological control.

On the "Puma" gamma unit with a ^{137}Cs radiation source with a dose rate of 2.26×10^{-5} A/kg, the animals were subjected to general uniform gamma irradiation at a dose of 7.5 Gy. A few minutes after radiation exposure, grade IIIB burn was simulated.

For treatment of combined radiation and heat injury of animals a vegetable origin preparation, purified turpentine (turpentine oil, produced by "Reaktiv", LLC, Russia), was used, which was applied in the doses of 0,2 ml; 0,4 ml; 0,6 ml; 1,0 ml. The drug was injected once subcutaneously under the wound surface of the affected area during the first 24 hours after radiation-thermal exposure.

The wound healing (granulation) efficacy of the drug was evaluated by observing control and treated animals, taking into account the severity of the course, healing time, and rejection of scabs from the burn eschar.

Results and Discussion. It was found that gamma-irradiation of white rats at the dose of 7.5 Gy (group 3) caused in them severe radiation sickness (figure 1-a), the characteristic features of which were the following: general depression and decreased locomotor activity; a significant decrease of feed and water consumption; coat rumpleness; the pallor of visible mucous membranes and ocular fundus (due to disruption of erythropoiesis); dark brown drying crusts in external eye corners and nasal passages (haemorrhagic syndrome); diarrhoea (Figure 1-b).

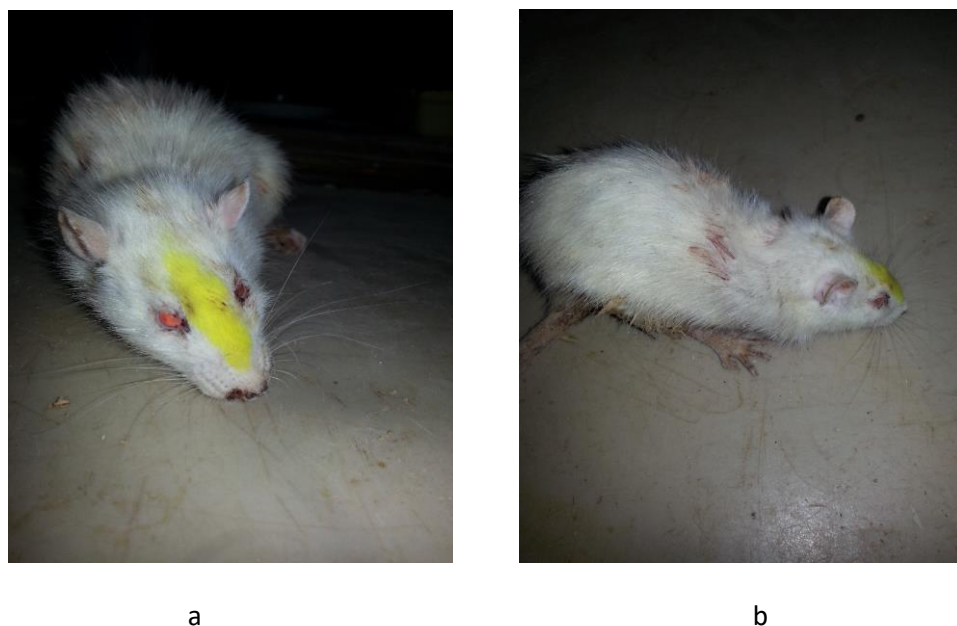


Figure 1: Appearance of white rats exposed to 7.5 Gy at 8 (a) and 11 (b) days after exposure

Animal mortality in the irradiation control group was 40% with an average life expectancy (ALE) of 13.0 days in the dead rats (Table 1).

Table 1: The indicators of the development and course of two-factor pathology caused by thermal trauma against a background of external gamma-exposure

Numbers of groups	Burn eschar formation` (day)	Burn eschar rejection (day)	Healing of burn wounds (day)	Fallen (heads)	ALE (day)
1	6-9	15-21	50-54	2	14
2	7-12	15-20	53-60	3	10
3	—	—	—	2	13
4	3-4	15-16	45-47	—	—
5	3-5	15-18	48-50	1	18
6	—	—	—	—	—

Infliction of thermal injury on white rats showed that the degree of burn injury was a function of the time the heated plate was held on the body surface. Thus, a 5-second contact of the heated object with the skin caused grade IIIA burn (Figure 2-a), which was characterized by the formation of a light brown eschar that detached after 15-16 days with subsequent epithelialization from the wound edges. The application of the heated plate for 8 seconds caused thermal damage characteristic of grade IIIB burns (Figure 2-b), which showed lesions not only of the skin with sebaceous and sweat glands and hair follicles, but also of subcutaneous tissue, as well as muscle and connective tissue. After 3 to 5 days, a dark brown to black necrotic eschar was formed. Under the above conditions of exposure to the thermal factor, the death of one animal on day 5 of the experiment was observed. A complete healing of burn wounds occurred on the 49th day after the thermal injury.

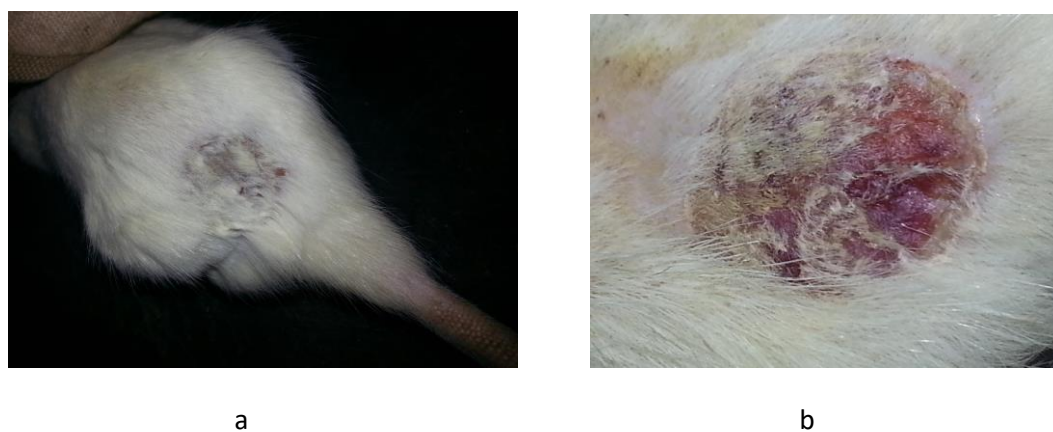


Figure 2: The condition of burn surfaces on the 8th day after thermal trauma (a - burn, exposure 5 sec; b - burn, exposure 8 sec).

Infliction of III-A degree burns on white rats against the background of radiation damage caused the death of two experimental animals at 14 days, the eschar formation occurred on 6-9 days, the eschar detachment - on 15-21 days, the complete wound healing - on the 50th day.

The gamma-irradiation of white rats at the dose of 7.5 Gy followed by deep thermal injuries (IIIB degree burns) had a worsening effect on the course of the burn disease which was manifested by 60% death of animals at ALE 10.0 days, later formation (7-12 days) of an eschar and its rejection on 15-20 days. A complete healing of burn wounds in surviving animals occurred by 53 days after exposure to radiation and thermal factors.

Thus, as a result of the first stage studies, the most adequate model of a combined radiation-thermal injury consisting of 7.5 Gy irradiation and IIIB degree burns was selected (the 2nd group of experiments).

During the next stage, studies were conducted to investigate the therapeutic effects of purified turpentine for the TRI treatment.

The rats subjected to the two-factor lesion and untreated (group 6) showed suppuration of the affected body area. The burn wound did not heal and the animals died as a result of wound sepsis. The first case of mortality in white rats exposed to radiation and heat injury was observed at day 4, while in the monoexposure (irradiation control) group it was not until day 9. Despite absolute mortality in the compared groups, mortality periods were 2-3 times longer (17, 17, 18, 24, 25 days) in the irradiated and heat-treated animals than in the control group (6, 7, 8, 9, and 38 days) with ALE 18.3 and 12.0 days, respectively. In animals of groups 2 and 4 treated with purified turpentine, in contrast to controls, the course of radiation and burn disease proceeded according to a modified (lightened) variant. The research results are presented in Table 2.

Table 2: Radioprotective effectiveness of purified turpentine in the TRI treatment by injecting it under the burned skin surface

Numbers	Terms of the	Indicator ($M \pm m$)	Survival	ALE
---------	--------------	-------------------------	----------	-----

of groups	experience	Complete eschar formation (days)	Complete eschar rejection (days)	Complete healing of burn wounds (days)	rate, %	(day)
1	Exp. 7.5 Gy + burn III B + treatment 0.2 ml	6.00 ± 0.40 n = 6	17.5 ± 0.33 n = 4	49 ± 0 n = 1	16.7	14.0
2	Exp. 7.5 Gy + burn III B + treatment 0.4 ml	5.83 ± 0.77 n = 6	15.83 ± 0.52 n = 6	36.6 ± 1.04 n = 5	83.3	24.0
3	Exp. 7.5 Gy + burn III B + treatment 0.6 ml	6.33 ± 1.19 n = 6	17.33 ± 0.41 n = 3	— n = 0	0	15.8
4	Exp. 7.5 Gy + burn III B + treatment 1.0 ml	7.17 ± 0.52 n = 6	17.83 ± 0.34 n = 6	40.5 ± 0.75 n = 4	66.7	20.5
5	Exp. 7.5 Gy + burn III B + lent. synth.	8.40 ± 0.67 n = 5	21.00 ± 2.12 n = 3	50.5 ± 2.12 n = 2	33.3	14.5
6	Exp. 7.5 Gy + burn	9.0 ± 0.0 n = 1	18.0 ± 0.0 n = 1	— n = 0	0	12.0
7	Burn III B	9.8 ± 1.4 n = 6	20.2 ± 0.66 n = 6	65.0 ± 1.8 n = 5	83.3	26.0
8	Exp. at 7.5 Gy	—	—	—	0	18.3
9	Biol. cont.	—	—	—	100	—

Note: n - number of animals, ALE - average life expectancy of fallen animals, area - γ -irradiation, lin. synth. - synthomycin liniment, tr. - treated, biol. contr. - biological control.

The data analysis in the table shows that purified turpentine has a radio- and heat-protective effect. The optimal therapeutic dose is 0.4 ml when injected once directly under the skin lesion. The process of burn eschar formation began on the 2nd day and in the control animals on the 6th day.

The complete formation of an eschar with clearly demarcated boundaries was observed on day 6 after TRI exposure (Figure 3) in rats of experimental groups 1-3, on day 7 in group 4, on day 8 in group 5, on day 9 in animals of control groups 6 and 7. Seven days after the start of the experiment, the death of two rats from group 3 was observed.

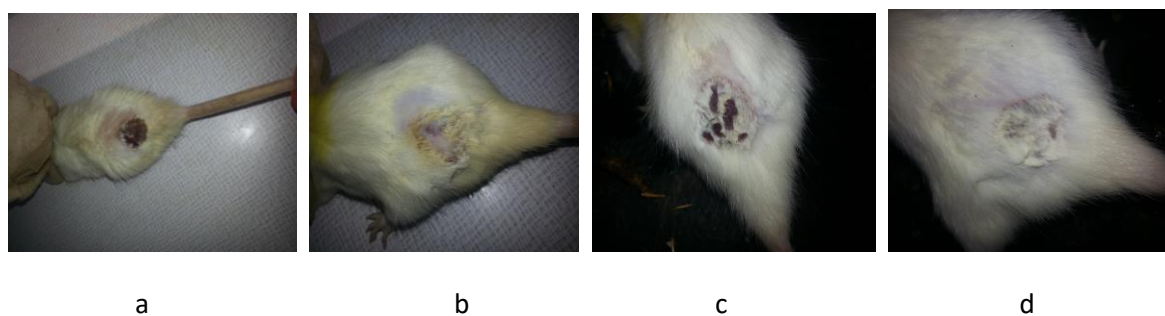


Figure 3: Formation of burn scabs on the 6th day after TRI (a - gamma-irradiation at 7.5 Gy + grade IIIB burn + treatment with 0.4 ml of purified turpentine; b - 7.5 Gy γ -irradiation + grade IIIB burn + treatment with 10% liniment of synthomycin; c - 7.5 Gy γ -irradiation + grade IIIB burn; d - grade IIIB burn without irradiation).

A visual assessment of the clinical condition of the animals on day 8 of the study indicated that behavioural reactions, the condition of visible mucous membranes, feed and water consumption, motor activity and other indicators were adequate in the animals of this group, while the control group rats showed depression, coat rumpleness, reduced motor activity and feed consumption. At this point in time, one animal from Group 1 died. White rats also suffered mortality: on day 9, one rat from group 5 died; on day 10, one rat from group 1 died; on day 12, one rat each from groups 3 and 5; and on day 14, one rat from group 5 died as well.

The rejection of burn eschars was observed on day 15 in three animals of group 2, on day 16 in one of group 2, on day 17 in two animals of groups 1, 2, 3 and 4, while it was completely retained in white rats of group 5 (Figure 4). Clinical examination recorded the death of one rat at day 18 of group 1 and one animal at day 20 of group 1 and group 4.

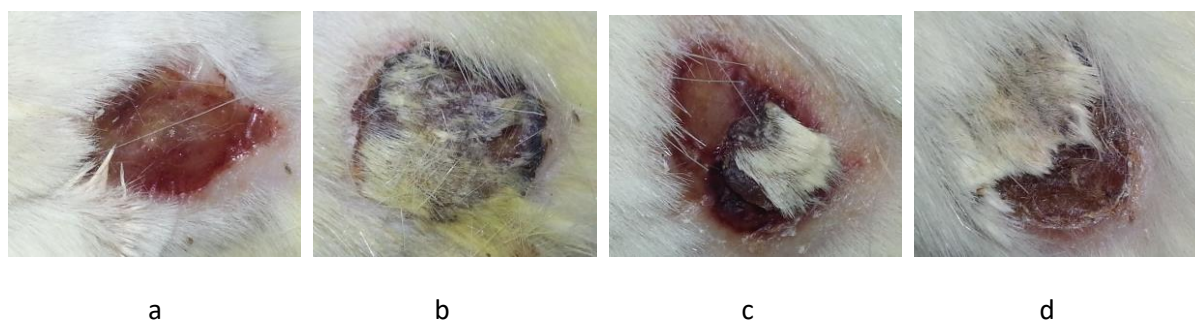


Figure 4: Condition of burn wounds at 17 days after irradiation and thermal trauma (a - gamma-irradiation at 7.5 Gy + grade IIIB burn + treatment with purified turpentine at a dose of 0.4 ml; b - 7.5 Gy γ -irradiation + grade IIIB burn + treatment with 10% liniment of synthomycin; c - 7.5 Gy γ -irradiation + grade IIIB burn; d - grade IIIB burn without irradiation).

It was established that on the 21st day of the experiment the burn eschars completely rejected in all animals of the 1st, 3rd and 4th groups, two animals of group 5, one of group 6, and five animals of group 7 (control group). In white rats treated with purified turpentine, a line of demarcation and granulation tissue appears around the affected areas on the 19th day after the start of treatment, while in controls it is observed on the 25th day. On day 23 of the experiment, three rats from group 3 and one rat from group 5 died, and one rat from group 2 died on day 24.

The rejection of a burn eschar in white rats of the control group with signs of wound suppuration in two animals of group 7, one of which died 26 days after the radiation-thermal action, was observed.

The main criterion for evaluating the efficacy of the drug in the treatment of both conventional and radiation-induced burns is the time of complete healing of the burn wounds. Against the background of the application of the proposed remedy, healing of a thermal (burn) injury was established in one animal from group 2. On the 31st day of the experiment, suppuration of the wound surface was observed in two rats of group 7 (control group).

The complete healing of burn wounds in animals treated with purified turpentine occurred on the 36th day after application of the combined lesion (group 2), healing on the 40th day was registered in four rats of group 4, while healing was not registered in any animal of group 7 at the given time of observation. On the contrary, suppuration was observed in one animal.

At 51 days, an absolute healing of burn wounds was observed in the entire population of rats of groups 1, 5, but not in animals of group 7.

In the control animals of group 7, healing of the burn wounds occurred on average on day 65 after the combined lesion.

CONCLUSION.

Thus, it was established experimentally that gamma-irradiation of white rats at the dose of 7.5 Gy causes in them severe acute radiation sickness, and the application of a metal plate heated to 190 °C for 5 and 8 seconds causes IIIA and IIIB degree burns respectively. It was shown that the degree of thermal lesions inflicted against the background of ionizing radiation influences the course and the outcome of radiation-thermal pathology that is accompanied by the reduced survival rate of experimental animals, decrease in the period of formation and rejection of a burn eschar, and healing of thermal lesions, i.e. the postulate of mutual aggravation of the two pathological factors is confirmed.

The use of purified turpentine as a biological preparation of plant origin for treatment of TRI animals when directly injected under the burned skin area provided a significant increase in the survival rate of the affected animals due to accelerated healing (granulation) of the affected tissue and inhibition of general organism intoxication.

Source of funding. The authors declare that they have no funding support for this study.

Conflict of interest. The authors declare that there is no known conflict of interest associated with this publication.

REFERENCES

- Vaiserman A, Koliada A, Zabuga O, and Socol Y (2018) Health Impacts of Low-Dose Ionizing Radiation: Current Scientific Debates and Regulatory Issues Dose-response a publication of International Hormesis Society 16(3) 1559325818796331.
- Wang H, Wei J, Zheng Q, Meng L, Xin Y, Yin X and Jiang X (2019) Radiation-induced heart disease: a review of classification, mechanism and prevention. International journal of biological sciences 15(10) 2128–2138.

- Rios C I, DiCarlo A L and Marzella L (2020) Cutaneous Radiation Injuries: Models, Assessment and Treatments. *Radiation research* 194(3) 310–313.
- Friedstat J, Brown D A and Levi B (2017) Chemical, Electrical, and Radiation Injuries. *Clinics in plastic surgery* 44(3) 657–669.
- Jeschke M G, van Baar M E, Choudhry M A, Chung K K, Gibran N S and Logsetty S (2020) Burn injury. *Nature reviews. Disease primers* 6(1): 11.
- Socol Y (2015) Reconsidering Health Consequences of the Chernobyl Accident. *Dose-response: a publication of International Hormesis Society* 13(1) dose-response 14-040 Socol.
- Gaynutdinov TR, Nizamov RN, Idrisov AM, Rakhmatullina GI and Guryanova VA (2021) Obtaining radioactivated strains of microorganisms and studying their antiradiation efficiency. *IOP Conf. Series: Earth and Environmental Science* 723: 042008.
- Proshkina E, Plyusnin S, Babak T, Lashmanova E, Maganova F, Koval L, Platonova E, Shaposhnikov M and Moskalev A (2020) Terpenoids as Potential Geroprotectors. *Antioxidants* 9(6): 529.
- Kuruba V and Gollapalli P (2018) Natural radioprotectors and their impact on cancer drug discovery. *Radiation oncology journal* 36(4): 265–275.
- Samarth R M, Samarth M and Matsumoto Y (2017) Medicinally important aromatic plants with radioprotective activity. *Future science OA* 3(4): FSO247.