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DEVELOPMENT AND EXPERIMENTAL ASSESSMENT OF A TERPENOID-BASED RADIOPROTECTIVE FORMULATION: RADIOPROTECTIVE AND RADIOTHERAPEUTIC PROPERTIES

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Introduction. The use of nuclear energy may give rise to emergency situations accompanied by the release of radioactive elements into the environment, potentially leading to radiation injuries to personnel of such enterprises or the entire population. In this context, the development of effective and safe approaches for protecting the body from radiation injuries remains highly relevant [1].
Objective. Study of the radioprotective properties of purified turpentine oil (PTO) and its combination with sunflower oil.
Materials and methods. At the first stage of research, the composition of PTO was determined and an optimal solvent was selected. The administration route (subcutaneous, intraperitoneal, intramuscular) of turpentine-oil solutions (at doses of 1806 mg/kg, 1290 mg/kg, 774 mg/kg, 516 mg/kg, and 2580 mg/kg) in 360 mice of both sexes (weighing 18–20 g) was established. At the second stage, the radioprotective efficacy of turpentine–oil solutions was evaluated in 50 white mice (70%, 50%, 30%, 20% turpentine-oil solutions were administered intramuscularly at doses of 1806 mg/kg, 1290 mg/kg, 774 mg/kg, 516 mg/kg 24 h before and after irradiation at a dose of 8.0 Gy ($LD_{100/30}$)). At the third stage, the optimal dose ensuring the greatest radioprotective efficacy was determined in 120 white mice of both sexes (irradiation at a dose of 7.7 Gy ($LD_{100/30}$); intramuscular administration of a 70% turpentine–oil solution at doses of 1806 mg/kg, 180.6 mg/kg, 90.3 mg/kg, 45.15 mg/kg, 22.57 mg/kg 72 h before and after irradiation). At the fourth stage, the radioprotective efficacy of 50% and 70% turpentine–oil solutions was evaluated in 36 outbred white rats of both sexes (irradiation at a dose of 9.3 Gy; after 3 days, a single subcutaneous administration of PTO at a dose of 258 mg/kg, anti-radiation serum at a dose of 50 mg/kg; intramuscular administration of a 70% turpentine–oil solution at a dose of 90.3 mg/kg, a 50% solution at a dose of 64.5 mg/kg). The content of malondialdehyde in the blood serum was determined on days 3, 5, 7, and 14 after irradiation. At the fifth stage, the optimal timing for the administration of the 70% turpentine–oil solution was determined in 80 white mice irradiated at a dose of 8.0 Gy. Statistical data analysis was performed using the GraphPadPrism v. 8.0 software package.
Results. Highly purified, high-oleic refined sunflower oil was identified as the optimal solvent for PTO. The preferred route of administration is intramuscular for turpentine–oil solutions and subcutaneous for PTO. The most pronounced radioprotective activity was observed for 70% and 50% turpentine–oil solutions administered as a single intramuscular injection 24 h before and after irradiation. To ensure 80% survival of lethally irradiated animals with a single prophylactic use and 60% survival with therapeutic use, intramuscular administration of the developed agent at doses of 90.3–180.6 mg/kg is required during the first 12 days before or during the first 4 days after lethal irradiation. The application of turpentine–oil solutions in various concentrations modified the course of the pathological process: by day 5 after treatment initiation, lethally irradiated animals showed a decrease in lipid peroxidation intensity.
Conclusions. A radioprotective agent has been developed that exerts both prophylactic and therapeutic effects by inhibiting lipid peroxidation products induced by ionizing radiation. The formulation is characterized by a a simple preparation technology, an effective route of administration, and an optimized ratio of components that ensures good absorption. As a result, a single intramuscular injection of the developed formulation can serve as an alternative to prolonged oral administration of terpenoid-based biologically active compounds.

Keywords: radiation sickness; lipid peroxidation products; radioprotectors; radiomitigators; purified turpentine oil; sunflower oil; survival rate

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РАЗРАБОТКА РАДИОЗАЩИТНОЙ КОМПОЗИЦИИ НА ОСНОВЕ ТЕРПЕНОИДОВ И ИЗУЧЕНИЕ ЕЕ РАДИОПРОТЕКТОРНОЙ, РАДИОТЕРАПЕВТИЧЕСКОЙ АКТИВНОСТИ В ЭКСПЕРИМЕНТЕ

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Введение. Использование атомной энергии может приводить к чрезвычайным ситуациям, сопровождающимся выбросами радиоактивных элементов в окружающую среду, в результате чего возможно возникновение лучевых поражений у персонала или населения. В этой связи актуальной задачей является поиск эффективных безопасных средств защиты от радиационных поражений организма.
Цель. Изучение радиозащитных свойств очищенного скипидара и его сочетания с подсолнечным маслом.
Материалы и методы. На первом этапе работы проведено определение состава и подбор растворителя для очищенного скипидара; установлен способ введения (подкожное, внутрибрюшинное, внутримышечное) скипидарно-масляных растворов (в дозах 1806, 1290, 774, 516, 2580 мг/кг) 360 мышам обоего пола (массой 18–20 г). На втором этапе проведена оценка радиозащитной эффективности скипидарно-масляных растворов на 50 белых мышах (внутримышечно вводили 70, 50, 30, 20% скипидарно-масляные растворы в дозах 1806, 1290, 774, 516 мг/кг за 24 ч до и после облучения в дозе 8,0 Гр ($ЛД_{100/30}$)). На третьем этапе определяли оптимальную дозу, обеспечивающую наибольшую радиозащитную эффективность, на 120 белых мышах обоего пола (облучение в дозе 7,7 Гр ($ЛД_{100/30}$), внутримышечное введение 70% скипидарно-масляного раствора в дозах 1806, 180,6, 90,3, 45,15, 22,57 мг/кг за 72 ч до и после облучения). На четвертом этапе проведена оценка радиозащитной эффективности 50 и 70% скипидарно-масляных растворов на 36 беспородных белых крысах обоего пола (облучение в дозе 9,3 Гр, через 3 сут однократное подкожное введение очищенного скипидара в дозе 258 мг/кг, противолучевой сыворотки в дозе 50 мг/кг; внутримышечное введение 70% скипидарно-масляного раствора в дозе 90,3 мг/кг, 50% раствора в дозе 64,5 мг/кг). Содержание малонового диальдегида в сыворотке крови определяли на 3, 5, 7, 14 сут после облучения. На пятом этапе работы определяли оптимальные сроки применения 70% скипидарно-масляного раствора на 80 белых мышах, облученных в дозе 8,0 Гр. Статистический анализ данных проведен с использованием пакета прикладной программы GraphPadPrism v. 8.0.
Результаты. Установлен оптимальный растворитель для очищенного скипидара на основе высокоочищенного высокоолеинового рафинированного подсолнечного масла; оптимальный путь введения для скипидарно-масляных растворов — внутримышечный, для очищенного скипидара — подкожный. Наиболее выраженной радиозащитной активностью обладали 70 и 50% скипидарно-масляные растворы при их однократном внутримышечном введении за 24 ч до и после облучения. Для обеспечения 80% выживаемости летально облученных животных при однократном профилактическом и 60% лечебном использовании необходимо внутримышечное введение разработанного средства в дозах 90,3–180,6 мг/кг в течение первых 12 сут до или в течение первых 4 сут после летального облучения. Применение исследуемых скипидарно-масляных растворов различной концентрации модифицировало течение патологического процесса: через 5 сут после начала лечения у летально облученных животных отмечали снижение интенсивности перекисного окисления липидов (ПОЛ).
Выводы. Разработано радиозащитное средство, оказывающее профилактическое и лечебное действие путем ингибирования продуктов перекисного окисления липидов, индуцированных ионизирующей радиацией. Разработанная композиция имеет простую технологию приготовления, эффективный способ введения и оптимальное соотношение компонентов, обеспечивающих хорошую рассасываемость, что позволяет заменить однократным внутримышечным введением разработанного средства длительное пероральное введение биологически активных веществ, содержащих терпеноиды.

Ключевые слова: лучевая болезнь; продукты пероксидации липидов; радиопротекторы; радиомитигаторы; очищенный скипидар; подсолнечное масло; выживаемость

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Финансирование: работа выполнена без спонсорской поддержки.

Соответствие принципам этики: все процедуры с модельными животными были проведены в соответствии с Правилами лабораторной практики и директивой Европейского парламента и Совета Европейского союза 2010/63/ЕС (2010 г.) о защите животных, используемых для научных целей. Проведение исследований одобрено на заседании локального этического комитета ФГБНУ «Федеральный центр токсикологической, радиационной и биологической безопасности» (протокол № 11 от 28.02.2023).

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INTRODUCTION

Ionizing radiation is currently used across various industrial and economic sectors, creating a potential risk of emergency situations [1–5]. In the event of an accident, priority is given to comprehensive measures aimed at mitigating the radiation hazard and ensuring the radiation safety of personnel in these sectors. Numerous

methods for the prevention and treatment of radiation injuries, intended to enhance the body's resistance to the effects of ionizing radiation, have been developed by both domestic and foreign researchers [6–15]. Research in this field is still underway.

Studies of the effect of the radioprotector indralin (B-190), administered alone or in combination with monizol, have shown that, as an α_1-adrenergic agonist, indralin induces a hypertensive response with the development of bradycardia in rabbits, decreases blood supply and spleen weight in rats and hybrid mice, and reduce markedly blood loss from wounds [16]. Chemical radioprotectors may have side effects, including toxicity to vital body systems, or demonstrate low efficacy. These limitations restrict the application of chemical radioprotectors and underscore the relevance of searching for safer radioprotectors of biological origin [17, 18]. Thus, peptides have been investigated in various preclinical models as biological radioprotectors, exerting their effect through free radical scavenging, alteration of cellular signaling pathways, and inhibition of cell apoptosis [19].

Nizamov et al. [20] reported the development of a biological preparation based on turmeric, using a culture fluid obtained during the cultivation of bifidobacteria as a suspension medium, designed for treating radiation injuries. In experimental studies, a single subcutaneous injection of a 0.5% turmeric suspension in a volume of 0.1 cm^3 increased the survival rate of lethally irradiated animals. Ivanov et al. proposed a preparation based on a mixture of biologically active honey-based substances, propolis, beebread, pollen, bee venom, bee brood, royal jelly, wax moth and their larvae, wax, dead bees, and grass meal, obtained by extraction with 70% ethanol. This preparation protected animals from lethal irradiation [21]. Nizamov et al. developed a feed additive aimed at enhancing the body's resistance, consisting of dead bees, Jerusalem artichoke tuber powder, grass meal, and a sorbent, which is recommended for use under combined irradiation [22]. Avilov et al. proposed a method for protecting the body from radiation injuries by a single subcutaneous administration of anti-radiation serum at doses of 100–125 mg/kg bw for young animals and 200–250 mg/kg for adults, administered within 10 days before and after irradiation [23].

Despite these achievements, the review of available literature on radioprotective agents reveals significant limitations of the aforementioned approaches. These include a delayed onset of biological effects following oral administration, the need for repeated dosing, and technological challenges associated with the production of the therapeutic agents, such as anti-radiation serum and the bifidobacteria–turmeric-based preparations.

Another line of research considers plant-derived terpenoids as potential agents with radioprotective activity. One such compound is miliacin (3-β-methoxy-Δ18-oleanene, a natural cyclic triterpenoid belonging to the group of natural cyclic triterpenoids and isolated from millet oil), which demonstrated antitoxic effects in [24, 25]. This compound was used as a protector against chromosomal aberrations induced by cyclophosphamide in mouse bone marrow cells [26].

Terpenoids derived from pine are known to exhibit anti-stress effects, realized through the inhibition of toxic radicals upon exposure of the body to ionizing radiation, chemical toxicants, and other pathogenic agents. Among them, turpentine oil (turpentine) is one of the most important terpenes used in medicine and veterinary medicine. It demonstrates a broad spectrum of biological activities, such as anti-inflammatory, analgesic, antibacterial effects, activation of granulation, stimulation of the central nervous system, immune- and hematopoiesis.

Our previous studies on the therapeutic efficacy of purified turpentine oil (a hydrocarbon-type compound $(C_5H_8)_n$) in white mice confirmed its high therapeutic efficacy, ensuring survival rates in the treatment of acute radiation sickness ranging from 60 to 100% in animals irradiated with gamma rays at doses 5.5–8.0 Gy [27]. In the development of therapeutic agents for protecting the body from radiation injury, it is essential to increase their efficacy while simplifying the treatment protocol, reducing its cost, and shortening duration.

The aim of this study was to investigate the radioprotective properties of purified turpentine oil (PTO), both alone and its combination with sunflower oil.

MATERIALS AND METHODS

The first stage of research consisted in the development of a radioprotective therapeutic agent based on PTO, i.e., a hydrocarbon-type compound $(C_5H_8)_n$, GOST 1571-82.

The selection of an optimal solvent (depot-forming agent) for turpentine oil was guided by such its properties, as insolubility in water, good solubility in alcohol and chloroform, miscibility in any ratio with ether, chloroform, gasoline, and vegetable oils. As a result, vegetable oils were chosen as the solvent, since other components listed above are highly toxic to the body.

The studies were conducted on experimental animals provided by the vivarium of the Federal Center for Toxicological, Radiation and Biological Safety (Kazan). The animals were kept under standard vivarium conditions (GOST 33215-2014). A 12-hour light cycle was maintained, with food and water provided *ad libitum*.

The experiment comprised five consecutive stages, outlined in Table 1.

Clinical observation of the animals was carried out over a 30-day period following irradiation. Survival rates and the mean life expectancy of deceased animals were recorded, along with the timing of edema formation and the rate and completeness of its resorption.

The concentration of malondialdehyde in the blood serum was determined on days 3, 5, 7, and

Table 1. Experimental design by stage

Stage number and title	Experimental conditions
1. Determination of an optimal composition and selection of potential solvents for PTO and their ratios. Study of optimal administration routes for turpentine-oil solutions and assessment of the body's response to these routes	The selection of potential solvents for PTO was carried out using sunflower oil (unrefined, refined, high-oleic refined sunflower oil "Solpro", TU 9141-006-70316851-2012, manufactured by Atkarsky Oil Extraction Plant (Russia)). High-oleic refined sunflower oil was selected as the solvent. Turpentine-oil solutions were prepared in various ratios (PTO / oil): S1 — 70% turpentine–oil solution; S2 — 50% turpentine–oil solution; S3 — 30% turpentine–oil solution; S4 — 20% turpentine–oil solution. The indicated turpentine-oil solutions, as well as PTO, were administered to 360 white mice (weighing 18–20 g) of both sexes, divided into experimental and control groups. Administration was performed subcutaneously, intraperitoneally, and intramuscularly at doses of 1806 mg/kg (S1), 1290 mg/kg (S2), 774 mg/kg (S3), 516 mg/kg (S4), and 2580 mg/kg (PTO)
2. Evaluation of the radioprotective efficacy of experimental compositions (in white mice) based on PTO and high-oleic refined sunflower oil, and selection of the most effective component ratios	Experiments were conducted on 50 white mice of both sexes (females n = 25; males n = 25), divided into 10 groups of 5 animals each. Animal irradiation was performed using a Puma gamma unit manufactured by the All-Russian Association Izotop (Russia) with a ^{137}Cs radiation source at a dose of 8.0 Gy ($LD_{100/30}$) with an exposure dose rate of 5.38 R/min (2.31×10^{-5} A/kg). Group 1 (n = 5; males — 3; females — 2) — 24 h before irradiation at a dose of 8.0 Gy, S1 was administered intramuscularly at a dose of 1806 mg/kg; Group 2 (n = 5; males — 3; females — 2) — 24 h before irradiation (8.0 Gy), S2 was administered intramuscularly at a dose of 1290 mg/kg; Group 3 (n = 5; males — 3; females — 2) — 24 h before irradiation (8.0 Gy), S3 was administered intramuscularly at a dose of 774 mg/kg; Group 4 (n = 5; males — 3; females — 2) — 24 h before irradiation (8.0 Gy), S4 was administered intramuscularly at a dose of 516 mg/kg; Group 5 (n = 5; males — 2; females — 3) — 24 h after irradiation at a dose of 8.0 Gy, S1 was administered intramuscularly at a dose of 1806 mg/kg; Group 6 (n = 5; males — 2; females — 3) — 24 h after irradiation at a dose of 8.0 Gy, S2 was administered intramuscularly at a dose of 1290 mg/kg; Group 7 (n = 5; males — 2; females — 3) — 24 h after irradiation at a dose of 8.0 Gy, S3 was administered intramuscularly at a dose of 774 mg/kg; Group 8 (n = 5; males — 2; females — 3) — 24 h after irradiation at a dose of 8.0 Gy, S4 was administered intramuscularly at a dose of 516 mg/kg; Animals in groups 1–8 received the studied agent in a volume of 0.1 cm^3; Group 9 (n = 5; males — 3; females — 2) — irradiation control (irradiation at a dose of 8.0 Gy); Group 10 (n = 5; males — 2; females — 3) — biological control (intact animals)
3. Determination of an optimal dose of 70% turpentine-oil solution providing the greatest radioprotective efficacy in the prophylactic and therapeutic application of the studied agent	Experiments were conducted on 120 white mice of both sexes (females n = 60; males n = 60) weighing 18–20 g, divided into 12 groups of 10 animals each. The animals were irradiated using a Puma gamma unit at an $LD_{100/30}$ dose (7.7 Gy) with an exposure dose rate of 5.38 R/min (2.31×10^{-5} A/kg). Group 1 (n = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 1806 mg/kg 72 h before irradiation; Group 2 (n = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 180.6 mg/kg 72 h before irradiation; Group 3 (n = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 90.3 mg/kg 72 h before irradiation; Group 4 (n = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 45.15 mg/kg 72 h before irradiation; Group 5 (n = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 22.57 mg/kg 72 h before irradiation; Group 6 (n = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 1806 mg/kg 72 h after irradiation; Group 7 (n = 10; males –5; females — 5) — intramuscular administration of S1 at a dose of 180.6 mg/kg 72 h after irradiation;

Table 1 (continued)

Stage number and title	Experimental conditions
	Group 8 (*n* = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 90.3 mg/kg 72 h after irradiation; Group 9 (*n* = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 45.15 mg/kg 72 h after irradiation; Group 10 (*n* = 10; males — 5; females — 5) — intramuscular administration of S1 at a dose of 22.57 mg/kg 72 h after irradiation; Animals in groups 1–10 received the studied agent in a volume of 0.1 cm^3; Group 11 (*n* = 10; males — 5; females — 5) — irradiation control (irradiation at a dose of 7.7 Gy); Group 12 (*n* = 10; males — 5; females — 5) — biological control (intact animals)
4. Evaluation of the radioprotective efficacy of the composition based on PTO and high-oleic refined sunflower oil in white rats	The experiments were conducted on outbred white rats of both sexes (females *n* = 18 and males *n* = 18) weighing 200 g, irradiated using a Puma gamma unit at a dose of 9.3 Gy with an exposure dose rate of 2.31×10^{-5} A/kg. Group 1 (*n* = 6; males — 3; females — 3) — 3 days after irradiation, a single subcutaneous injection of PTO was administered at a dose of 258 mg/kg; Group 2 (*n* = 6; males — 3; females — 3) — 3 days after irradiation, a single intramuscular injection of 70% turpentine-oil solution was administered at a dose of 90.3 mg/kg; Group 3 (*n* = 6; males — 3; females — 3) — 3 days after irradiation, a single intramuscular injection of 50% turpentine-oil solution was administered at a dose of 64.5 mg/kg; Group 4 (*n* = 6; males — 3; females — 3) — 3 days after irradiation, a single subcutaneous injection of the comparison drug — anti-radiation serum (ARS) with known radioprotective efficacy — was administered at a dose of 50 mg/kg (0.2 cm^3) [23]; Group 5 (*n* = 6; males — 3; females — 3) — irradiation control (irradiation at a dose of 9.3 Gy); Group 6 (*n* = 6; males — 3; females — 3) — biological control (intact animals). Animals in groups 1–3 received the studied agent in a volume of 0.1 cm^3. The level of lipid peroxidation products — malondialdehyde — was studied [28]
5. Determination of optimal timing for administration of the studied agent (S1) for prophylactic and therapeutic purposes	Experiments were conducted on 80 white mice of both sexes (females *n* = 40; males *n* = 40) weighing 18–20 g, divided into 16 groups of 5 animals each. Irradiation of animals in all groups at a dose of 8.0 Gy was performed using a Puma gamma unit. White mice in groups 1–8 received S1 intramuscularly at a dose of 1806 mg/kg for prophylactic purposes 1, 2, 4, 6, 8, 10, 12, and 14 days before lethal irradiation. Animals in groups 9–16, under similar conditions, received S1 at the same dose 1, 2, 4, 6, 8, 10, 12, and 14 days after lethal irradiation (for therapeutic purposes)

Table compiled by the authors based on their own data

Note: S1 — 70% turpentine-oil solution; S2 — 50% turpentine-oil solution; S3 — 30% turpentine-oil solution; S4 — 20% turpentine-oil solution.

14 after irradiation and expressed as the equivalent amount of MDA, using a molar extinction coefficient of 1.56×10^5 (mol/L) × cm^{-1}. The absorption spectrum of the thiobarbituric acid-colored product was recorded using an SF-46 double-beam spectrophotometer (Russia).

Statistical data analysis was performed using the GraphPadPrism v. 8.0 software package. The reliability of the data obtained was determined using Student's *t*-test with Bonferroni correction.

RESULTS AND DISCUSSION

During the selection of an optimal solvent for PTO, it was established that, among the tested turpentine–oil formulations prepared with sunflower oils of varying degrees of refinement, solutions based on highly purified, high-oleic refined sunflower oil (Solpro) were the most suitable in terms of tolerability and route of administration. When administered intramuscularly, these solutions did not cause edema formation at the injection site.

The results of studies on the application of turpentine–oil solutions containing highly purified high-oleic sunflower oil are presented in Table 2.

Observations of the clinical condition of white mice over a 30-day period showed (Table 2) that intraperitoneal administration of all studied turpentine–oil solutions (S1, S2, S3) and native PTO resulted in mortality in all experimental groups, with the exception of the group (50% of animals survived) receiving turpentine–oil solution S4. All mice that received intraperitoneal injections of native (undiluted) PTO died on days 1–2; animals that received S1, S2, and S3 died on days 2–5 in the setting of diffuse edema and inflammation reaction at the injection site. In contrast, intraperitoneal administration of S4 led to a 50% mortality rate in white mice on days 14 and 17 after administration of the test solution.

Table 2. Results of applying turpentine-oil solutions administered to white mice

The therapeutic agent under study	Route and site of administration	Number of deceased animals, abs	Survival rate, %
S1 (70% turpentine–oil solution)	subcutaneously in the back area	–	100
	intraperitoneally in the abdominal area	8	0
	intramuscularly in the thigh area	–	100
S2 (50% turpentine–oil solution)	subcutaneously in the back area	–	100
	intraperitoneally in the abdominal area	8	0
	intramuscularly in the thigh area	–	100
S3 (30% turpentine–oil solution)	subcutaneously in the back area	–	100
	intraperitoneally in the abdominal area	8	0
	intramuscularly in the thigh area	–	100
S4 (20% turpentine–oil solution)	subcutaneously in the back area	–	100
	intraperitoneally in the abdominal area	4	50
	intramuscularly in the thigh area	–	100
Purified turpentine oil	subcutaneously in the back area	–	100
	intraperitoneally in the abdominal area	8	0
	intramuscularly in the thigh area	8	0

Table compiled by the authors based on their own data

Note: "–" — absence of deceased animals; 0 — absence of surviving animals.

Intramuscular administration of S1, S2, S3, and S4 to mice was associated with edema at the injection site on day 6, with no lameness observed in the animals. On day 8 of the study, animals in three groups (receiving S2, S3, and S4) showed slight inflammation at the injection site, while in animals receiving S1, no pronounced inflammation was noted. On day 12, the condition of animals that received turpentine-oil solutions did not differ significantly from animals in the control group.

In all animals, intramuscular administration of native PTO resulted in edema in the thigh area and lameness on day 6. On days 7–8, the lameness progressed, inflammation at the injection site became more pronounced (palpation revealed edema of a dense consistency), and movements in the limb were limited. On day 20 after drug administration, muscle atrophy of the specified limb was observed in the animals. In the setting of progressive weight loss, atrophy, and necrosis of the limb, all mice that received intramuscular injections of native PTO died between days 21 and 27 of the experiment.

Upon subcutaneous administration of PTO and its mixtures with oil at 20%, 30%, 50%, and 70% concentrations, animals in all experimental groups developed localized edema in the back area with scab formation at the injection site during the first 7–8 days post-injection. By day 14 of observation, animals that received 20%, 30%, and 50% turpentine–oil solutions showed scab detachment accompanied by hair regrowth at the site. In animals that received PTO, mild swelling was observed at the injection site by this time point, which had completely disappeared by day 25. A slight edema with clear boundaries was recorded with the administration of the 70% turpentine–oil solution. From days 15 to 17, in animals that received S2, S3, and S4, the appearance of a minor ulcer in the back area was observed; by day 20, the ulcer disappeared followed by scab formation and its detachment by day 25 with the onset of hair regrowth. On day 20, in animals that received subcutaneous injections of the 70% turpentine–oil solution, a bright red scab was observed, which detached by day 27 with restoration of hair cover. On days 26–28 of the experiment and until the end of the observation period, animals in all groups showed the absence of all previously existing signs of skin damage.

The obtained experimental results on assessing the efficacy of different administration routes for native turpentine oil (PTO) and its various combinations with sunflower oil at 20%, 30%, 50%, and 70% concentrations indicate that PTO is most suitable for subcutaneous administration, as it is associated with weight gain in mice. Intraperitoneal administration of

PTO at this dose is toxic, while intramuscular administration leads to tissue necrosis at the injection site and results in complete mortality. Turpentine–oil solutions should be administered intramuscularly: this administration route allows rapid absorption of the solutions without toxic effects. Subcutaneous administration of turpentine–oil solutions causes a well-demarcated aseptic abscess, which resolves more slowly compared to that observed following PTO administration. Intraperitoneal administration of these solutions is also associated with toxic effects.

Therefore, the optimal route of administration for turpentine–oil solutions is intramuscular, whereas subcutaneous administration should be preferred for PTO.

Studies aimed at determining the optimal ratio of PTO to highly purified high-oleic sunflower oil (Solpro) for good resorption of the administered composition identified the 70:30 (S1) ratio as the most optimal.

The results of the second research stage on studying the radioprotective efficacy of compositions based on PTO and sunflower oil are presented in Table 3.

It was established that turpentine–oil solutions S1 (70%) and S2 (50%) exhibit radioprotective activity both after a single intramuscular injection 24 h before irradiation and when administered 24 h after irradiation. The highest survival rate of lethally irradiated animals was observed with prophylactic administration of S1 (80%) and with therapeutic administration (60%). The optimal component ratio in both routes of drug administration was 7:3 (PTO:SO), corresponding to S1 (PT:SO = 70:30). Any deviations from this ratio resulted in decreased radioprotective efficacy.

During the third stage of the study aimed at determining the optimal dose for maximal radioprotective efficacy under prophylactic (before irradiation) and therapeutic (after irradiation) conditions, S1 demonstrated the maximum radioprotective effect (60–80% survival) at doses of 1806 mg/kg, 180.6 mg/kg, and 90.3 mg/kg. Administration of S1 at a dose of 45.15 mg/kg led to a decrease in the therapeutic effect (20% survival). The optimal therapeutic and prophylactic doses were found to be 90.3 and 180.6 mg/kg, administered in volumes of 0.25 and 0.5 cm^3/kg.

The results of the fourth stage aimed at evaluating the anti-radiation efficacy of the studied preparations in white rats are presented in Table 4.

It was established that rats in group 5 (irradiation control) exhibited the bone marrow form of acute radiation sickness of an extremely severe degree, with 100% animal mortality and the mean life expectancy of 8.2 days. The studied solutions reduced the severity of acute radiation sickness, transitioning the extremely severe degree of the disease to a severe degree and significantly increasing the mean life expectancy of the deceased animals.

In animals of group 3 (S2 administration), a severe degree of the bone marrow form of acute radiation sickness was observed; the survival rate under these

Table 3. Survival rates of white mice irradiated at a dose of 8.0 Gy when using compositions based on purified turpentine oil and sunflower oil

Group	Component ratio of turpentine–oil solutions (PTO:SO)	Time points before and after administration of turpentine–oil solutions	Test results		
			Animal mortality, abs count	Surviving animals	
				abs count	%
1	70:30	24 hours before irradiation	1	4	80
2	50:50	24 hours before irradiation	2	3	60
3	30:70	24 hours before irradiation	3	2	40
4	20:80	24 hours before irradiation	4	1	20
5	70:30	24 hours after irradiation	2	3	60
6	50:50	24 hours after irradiation	3	2	40
7	30:70	24 hours after irradiation	4	1	20
8	20:80	24 hours after irradiation	4	1	20
9 irradiation control	not used	administration was not performed	5	0	0
10 biological control	not used	administration was not performed	–	5	100

Table compiled by the authors based on their own data

Note: PTO — purified turpentine oil; SO — high-oleic refined sunflower oil; 0 — absence of surviving animals; "–" — absence of animal mortality.

experimental conditions was 50%, with the mean life expectancy of 11.7 days.

The application of PTO and S1 (groups 1 and 2) exerted a modifying effect on the course of the bone marrow form of acute radiation sickness, reducing its severity from extremely severe to severe, increasing the survival rate of lethally irradiated animals to 66.7% and the mean life expectancy to 12.5–13.5 days compared to the irradiated control.

The anti-radiation serum used in the experiment as a comparison drug exerted a radiotherapeutic effect, also mitigating the course of acute radiation sickness by transitioning the extremely severe degree of the disease to a severe degree. However, the survival rate in this group was significantly lower than that following the administration of PTO and turpentine–oil solutions (groups 1 and 2), amounting to 50% with the mean life expectancy of 13 days.

The clinical condition of intact animals in group 6 did not change throughout the entire experimental period. The animals were active, and their appetite, mobility, reaction to external stimuli, and skin condition were normal. No cases of mortality were observed.

Thus, the results of assessing the radioprotective efficacy of the studied preparations in white rats showed that PTO and its combination with sunflower oil (S1) exerted a mitigating effect on the course of the bone marrow form of acute radiation sickness. These interventions reduced condition severity from extremely severe to severe, increased the survival rate of lethally irradiated animals (groups 1 and 2) by 66.7%, and extended the mean life expectancy to 12.5–13.5 days, compared to complete mortality and the 8.2-day mean life expectancy in the irradiation control group (group 5).

The results of studies on lipid peroxidation activity under the influence of the tested agents are presented in the figure.

The course of acute radiation sickness in white rats was accompanied by a drastic increase in the intensity of lipid peroxidation processes. Already on day 3 after irradiation, the MDA concentration in the blood of irradiated rats (group 1) showed a twofold increase ($p < 0.001$), gradually rising and reaching its maximum value by day 14 at a level of 10.14 ± 0.58 nmol/mL, compared to 4.64 ± 0.08 nmol/mL in intact animals.

The studied agents — PTO and turpentine–oil solutions of various concentrations (in animals from groups 1, 2, and 3) and anti-radiation serum (group 4) — modified the course of the pathological process. Thus, five days after treatment initiation in lethally irradiated animals, a prolonged decrease in the intensity of lipid peroxidation was noted compared to animals in the “irradiation control” group, which accounted for the increase in the survival rate of animals in these groups.

The results of the fifth stage of research, aimed at determining the optimal timing for administration of the 70% turpentine–oil solution, showed that the highest prophylactic (80% survival) and therapeutic (60% survival) effects were achieved with a single intramuscular administration of the agent to animals at doses of 90.3 and 180.6 mg/kg within 12 days preceding irradiation and within the first 4 days after irradiation.

Thus, the use of optimal administration routes for PTO (subcutaneous) and the turpentine–oil solution

Table 4. Survival rates of white rats irradiated at a dose of 9.3 Gy following administration of the developed solutions

Group	Experimental conditions	Severity of acute radiation sickness	Average life expectancy, days	Survival rate, %
1	Irradiation at a dose of 9.3 Gy + single subcutaneous administration of PTO at a dose of 258 mg/kg	severe	12.5	66.7
2	Irradiation at a dose of 9.3 Gy + single intramuscular administration of S1 at a dose of 90.3 mg/kg	severe	13.5	66.7
3	Irradiation at a dose of 9.3 Gy + single intramuscular administration of S2 at a dose of 64.5 mg/kg	severe	11.7	50
4	Irradiation at a dose of 9.3 Gy + single subcutaneous administration of ARS at a dose of 50 mg/kg	severe	13	50
5	Irradiation at a dose of 9.3 Gy, no treatment administered (irradiation control)	extremely severe	8.2	0
6	No irradiation or treatment was administered (biological control)	intact animals survived until the end of the experiment		100

Table compiled by the authors based on their own data

Note: PTO — purified turpentine oil; ARS — anti-radiation serum; S1 — 70% turpentine–oil solution; S2 — 50% turpentine–oil solution.

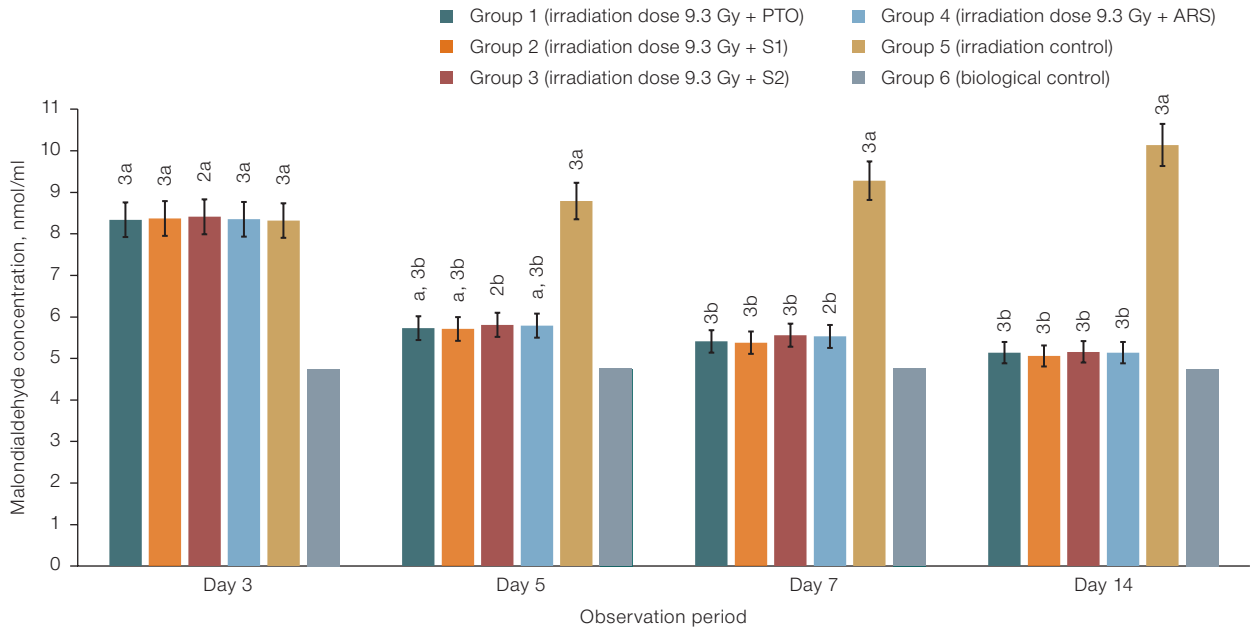

Figure prepared by the authors based on experimental results

Fig. Malondialdehyde content in the blood serum of white rats irradiated and treated with turpentine–oil solutions: PTO — purified turpentine oil; ARS — anti-radiation serum; S1 — 70% turpentine–oil solution; S2 — 50% turpentine–oil solution; a — statistically significant differences compared to biological control (a — $p < 0.05$; 2a — $p < 0.01$; 3a — $p < 0.001$); b — statistically significant differences compared to irradiation control (b — $p < 0.05$; 2b — $p < 0.01$; 3b — $p < 0.001$)

(intramuscular) prevents the development of a demarcation effect, ensures effective direct action on the biomembranes of radiation-damaged cells, stabilizes their functional state, and inhibits the pathological biochemical effects of toxic agents. Moreover, this approach simplifies the technology for obtaining the biological preparation and replaces the need for multiple, prolonged oral administration of biologically active substances containing terpenoids with a single intramuscular parenteral injection.

The use of PTO in combination with highly purified refined sunflower oil exerts both radioprotective and radiotherapeutic effects on the irradiated organism, primarily by inhibiting the formation of toxic lipid peroxidation products. As demonstrated in previous studies [17, 18, 27], these products cause apoptosis of immunocompetent cells (lymphocytes), leading to post-radiation mortality. Suppression of lipid peroxidation formation under the influence of the terpene-based mixture (turpentine oil and sunflower oil) enhances the body's resistance to the damaging effects of ionizing radiation.

CONCLUSION

1. The conducted study aimed at evaluating the body's response to the administration of compositions containing PTO and sunflower oils in different ratios and with varying degrees of refinement, their optimal ratio was established as 7 parts PTO to 3 parts highly purified high-oleic refined sunflower oil. The developed composition, when administered as a single intramuscular injection, does not induce a demarcation effect at the injection site and demonstrates good absorption, thereby enabling direct action on the biomembranes of radiation-damaged cells.

2. The established composition and component ratio of turpentine–oil solutions enable the achievement of pronounced radioprotective and radiotherapeutic effects, while simplifying both the preparation and administration process. Specifically, the developed composition replaces multiple and prolonged (over 30 days) oral administration of biologically active substances with a single intramuscular injection.

3. A single intramuscular injection of the developed 70% turpentine–oil solution to lethally irradiated animals at doses of 90.3 and 180.6 mg/kg 1–12 days before or 1–4 days after irradiation ensured an 80% and 60% survival rate of irradiated animals under the conditions of prophylactic and therapeutic use, respectively.

4. Intramuscular administration of the developed radioprotective composition (70% turpentine) inhibits the formation of lipid peroxidation products. These products cause apoptosis of immunocompetent cells — lymphocytes — leading to post-radiation mortality. By inhibiting through terpene components, the composition increases survival rates following exposure to ionizing radiation.

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