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ORIGINAL ARTICLE

Enhanced Radiosensitivity with *Hypericum Perforatum* in Radiation-induced Radiodermatitis Rat Model

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OBJECTIVE

Radiation dermatitis is one of the most common radiotherapy-related side effects. There is no gold standard treatment for this side effect, which drastically affects patients' quality of life. Nevertheless, patients often use over-the-counter products. *Hypericum perforatum* (St. John's wort) is one of the most commonly used homemade remedies. In this work, we investigated whether *Hypericum perforatum* oil augments radiation sensitivity in a rat radiodermatitis model.

METHODS

Radiation dermatitis was induced in 16 male Sprague-Dawley rats by administration of single fraction 30 Gy external radiotherapy. Rats were randomly assigned to control and *Hypericum perforatum* oil-treated arms. Starting on the third day before and up to 14 days after radiotherapy, the *Hypericum perforatum* oil arm was treated with 100 µl of *Hypericum perforatum* oil 3 times a day. Rats were observed for two weeks to assess acute skin changes, and irradiated rat skin samples were examined histomorphologically by scoring the presence of scarring, the severity of inflammation, and the extent of inflammation.

RESULTS

More skin reactions were observed in the *Hypericum perforatum*-treated arm. In the histomorphological examination, the *Hypericum perforatum* oil-treated arm had higher scores than the control arm in terms of the presence of scarring, the severity of inflammation, and the extent of inflammation.

CONCLUSION

Our results showed that *Hypericum perforatum* oil exacerbated radiation dermatitis, and caution should be exercised while recommending it.

Keywords: Animal model; *hypericum perforatum*; radiation dermatitis; radiosensitizer.

INTRODUCTION

Radiation dermatitis (RD) refers to a series of skin alterations caused by radiation exposure and is observed in around 95% of patients receiving radiotherapy (RT). [1] RD is characterized by acute and chronic skin changes. Its findings are erythema, dry desquamation, itching, wet desquamation, necrosis, and infection. The severity of RD is measured by various scoring systems used in the clinic, and treatment recommendations are

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made accordingly.[2] General skin care recommendations, moisturizing with hydrophilic moisturizers, topical corticosteroids, topical/systemic antibiotics, silicone foam bandages, and laser therapy are recommended for treatment.[3] Although there is no gold standard for its treatment, patients often use traditional methods.[4] Given that traditional techniques are typically derived from herbal sources, individuals may choose not to disclose their use to healthcare professionals on the assumption that they are devoid of any adverse effects.[5]

Hypericum perforatum (HP), also referred to as St. John's Wort, is a perennial plant that has its origins in Europe, West Asia, and North Africa. It has gained significant popularity for its extensive utilization in traditional ointments.[6] Especially oil-based HP preparations are frequently used in various skin inflammations, burns, wounds, and scars.[7] Also, in Turkish folk medicine, the utilization of olive oil macerate derived from flowering herbs is a commonly used home remedy for the treatment of gastrointestinal ailments as well as other dermatological conditions such as skin inflammations, wounds, and burns.[8] It is also an alternative method for the treatment of depression and mood disorders.[9] Hyperforin is the compound accountable for its anti-inflammatory, antibacterial, and keratinocyte differentiation properties.[10] It also possesses anti-cancer properties and may inhibit cancer invasion and metastasis.[11] The many properties of HP could render it an optimal therapeutic agent for the management of RD, a condition commonly observed in individuals with cancer. Hence, Franco et al.[12] assessed HP wort in a prospective trial on head and neck cancer patients and showed it was safe and had therapeutic benefits for RD.[13]

In addition to the therapeutic effects of HP, it also has photosensitivity-enhancing effects due to hypericin.[14] Hypericin has been found to exhibit absorption of UVA radiation at a wavelength of 300 nm, as well as absorption of visible light within the range of 550 to 590 nm. This absorption behavior is responsible for inducing photosensitizing effects.[10] This phenomenon is called "hypericism." There are studies reporting the increased therapeutic effects of HP with photodynamic therapy in bladder cancer and anaplastic thyroid cancer.[15,16] Again, studies on human melanoma cells and human skin have reported increased phototoxicity with ultraviolet light and solar-simulated radiation.[17–19] Although the photosensitizing effects of HP have been widely studied, there are merely two case reports in the literature that address the possibility of enhanced radiation sensitivity.[20,21] As of yet, there are no pre-clinic or prospective trials on this issue. In this study, we investigated the possible radiation sensitization-enhancing effect of prophylactic and simultaneous use of HP oil in a radiation-induced rat radiodermatitis model.

MATERIALS AND METHODS

Animals

All animal experiments were performed in accordance with Turkish laws on animal welfare and approved by the local ethics board (approval number: 428). To establish the rat RD model, 16 male Sprague-Dawley rats (12-week-old, 200–250 g) were used, and these rats were obtained from Kobay Laboratory, Ankara-Turkey. The experimental animals were housed in artificially lit rooms with a 12-hour light/dark cycle and temperatures ranging from 20°C to 24°C until euthanasia. The standard rat diet and water were supplied *ad libitum*. Rats were anesthetized with an intraperitoneal ketamine/xylazine cocktail before RT administration (90/10 mg/kg).

Hypericum Perforatum Oil Application

A quantity of 50 grams of HP was placed in a clear glass jar together with 500 ml of olive oil. The container was then exposed to sunlight for a duration of 12 hours each day for a period of 4 weeks throughout the summer season. Following the acquisition of HP oil, rats were randomized to control (n=8) and HP oil-treated (n=8) arms at the start of the experiment, and a 3×3 cm patch of their backs was shaved (Figs. 1, 2). The HP oil arm was treated with 100 µl of HP oil three times a day, starting on the 3rd day before RT. Treatment was continued up to the 14th day following RT.

Radiation-induced Dermatitis Model and Sacrification

RT was administered in a single fraction of 30 Gy at a dose rate of 600 MU with 4 MeV electrons under anesthesia (Fig. 2). Electrons were used only to irradiate the skin and protect the organs at risk underneath. The RT application was performed using the Varian Clinac DHX (Varian Medical System, Palo Alto, California, USA) linear accelerator at Hacettepe University Department of Radiation Oncology. Two animals allocated to the HP oil-treated group succumbed to problems related to anesthesia. Skin changes during the two weeks after RT were assessed using a semi-quantitative skin damage score in 14 animals (Table 1).[22] The rats were euthanized after two weeks, and skin samples were harvested.

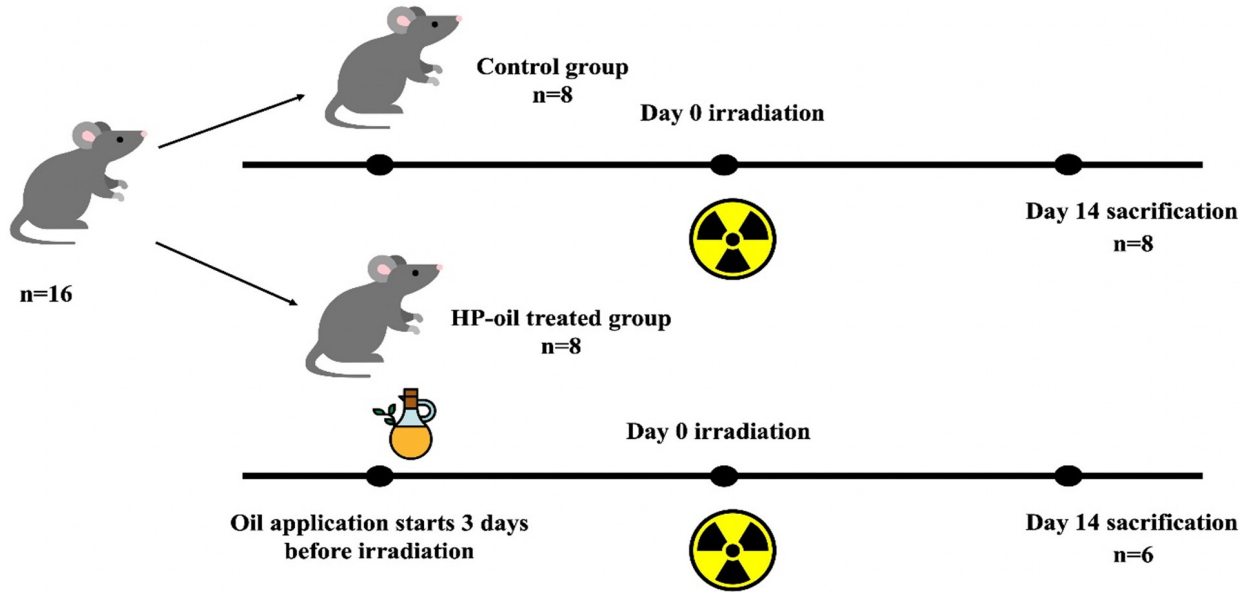

Fig. 1. Experimental design. The study involved a total of 16 rats, which were randomized into 8 control groups and 8 groups treated with *hypericum perforatum* (HP). The application of HP oil began three days before to the RT in the group treated with HP oil. During radiotherapy (RT), two rats from the group treated with HP oil succumbed to problems related to anesthesia. The sacrification was performed on the 14th day following the RT procedure.

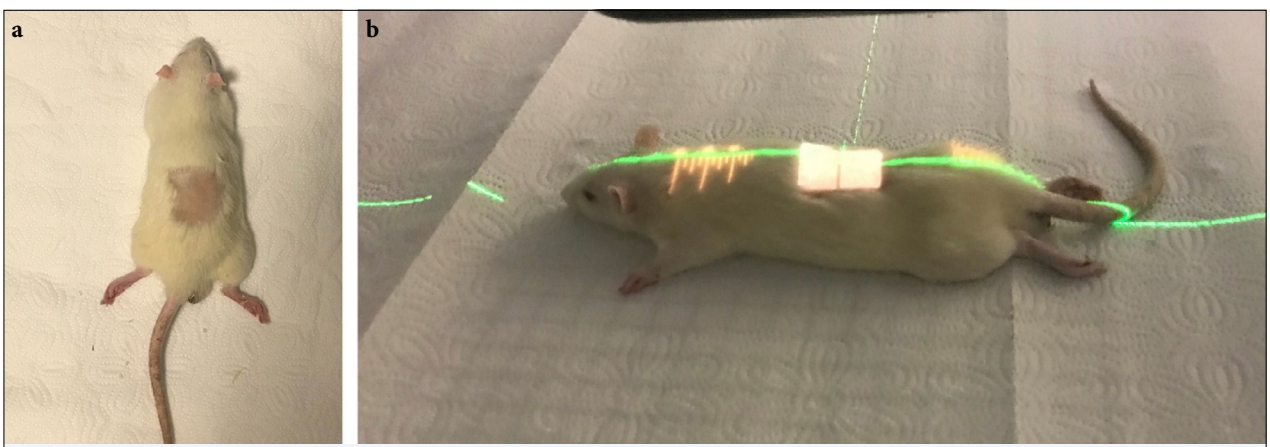

Fig. 2. (a) A patch measuring 3×3 cm after shaving on the dorsal area of each rat's back. After shaving, 100 µl of HP oil was applied three times daily, commencing on the third day prior to radiation therapy started in HP oil-treated arm. (b) Treatment position of the rat.
HP: *Hypericum perforatum*.

Histopathology

Irradiated rat skin samples were examined histomorphologically. The tissues were fixed with 4% paraformaldehyde overnight; paraffin blocks were prepared and stained with hematoxylin & eosin, and sections of 4 µm were cut. The stained preparations were evaluated by the pathologist using a light microscope by scoring the presence of scarring, and the severity and extent of inflammation. The presence of scarring was categorized as present, absent, and focal. The severity of inflammation was scored as 1, 2, and 3 as no/mild, moderate, and severe, respectively. The extent of inflammation was scored as 1, 2, 3, 4, and 5 as <10%, 11–25%, 26–50%, 51–75%, and >75% inflammation prevalence, respectively.

Statistical Analysis

Statistical analyses were performed with SPSS software version 23 (SPSS Inc., Chicago, IL, USA). De-

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Table 1 Semi-quantative skin damage scores

Score	Skin changes
1.0	No effect
1.5	Minimal erythema, mild dry skin
2.0	Moderate erythema, dry skin
2.5	Marked erythema, dry desquamation
3.0	Dry desquamation, minimal dry crusting
3.5	Dry desquamation, dry crusting, superficial minimal scabbing
4.0	Patchy moist desquamation, moderate scabbing
4.5	Confluent moist desquamation, ulcers, large deep scabs
5.0	Open wound, full thickness skin loss
5.5	Necrosis

scriptive statistics were presented as median and minimum–maximum. Differences between groups were analyzed with the Mann-Whitney U and the chi-square test. An overall 5% type-I error level was used to infer statistical significance.

RESULTS

Post-radiation Skin Damage Score

The HP oil-treated group (n=6) and the control group (n=8) were observed for two weeks to assess acute skin changes after RT. In both groups, skin reactions increased gradually over the first 14 days. On the 14th day, the maximum dermatitis score recorded in the HP oil group was 4 (patchy moist desquamation), while the highest score recorded in the control group was 2.5 (dry desquamation). Figure 3 presents the skin damage scores for each group's minimum, maximum, and median values throughout a 14-day period. Accordingly, the difference in skin reactions between the two arms was evident (p=0.001). More skin reactions were observed in the HP oil-treated arm.

Histopathological Evaluation

All samples treated with HP oil (n=6) showed signs of diffuse scarring, severe inflammation (score 3), and inflammation with a prevalence of at least 50%. Looking at the extent of inflammation scores in more detail, 4 (66%) had >75% inflammation, and 2 (33%) had 51–75% inflammation. In the control arm, on the other hand, scarring was absent in 5 (62%), and in 3 (37%) cases only focal scarring was observed. Inflammation was absent or mild in 50% of cases (n=4), with an extent of <10%. In the other half of the cases (n=4), inflammation was moderate, with an extent ranging from 11% to 25% (Fig. 4). Harvested skins treated with HP oil had statistically higher scores than the control arm in terms of the presence of scarring, and the severity and extent of inflammation (p=0.001, p=0.001, p=0.003, respectively).

DISCUSSION

To the best of our knowledge, this is the first preclinical study showing the radiation-enhancing effect of HP oil in the RD model. In our study, whether HP oil could prevent RD and diminish RD-related symptoms in prophylactic and post-RT therapeutic use was investigated. However, we observed increased RD and increased

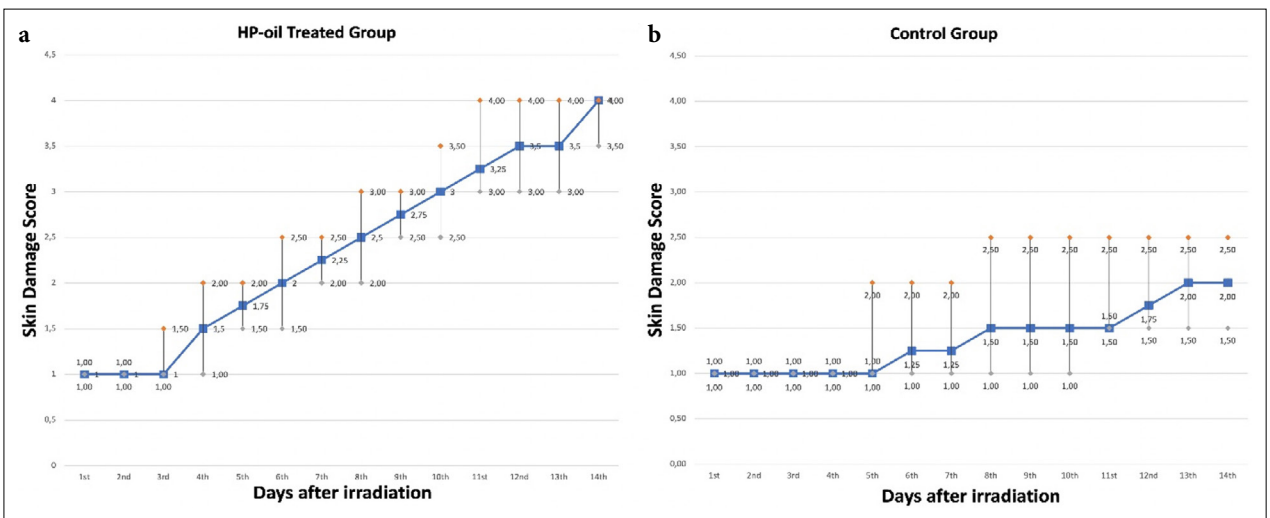

Fig. 3. Median (minimum-maximum) skin damage scores over a 14-day period for each group (p<0.05). (a) *Hypericum perforatum* oil-treated group (b) control group.

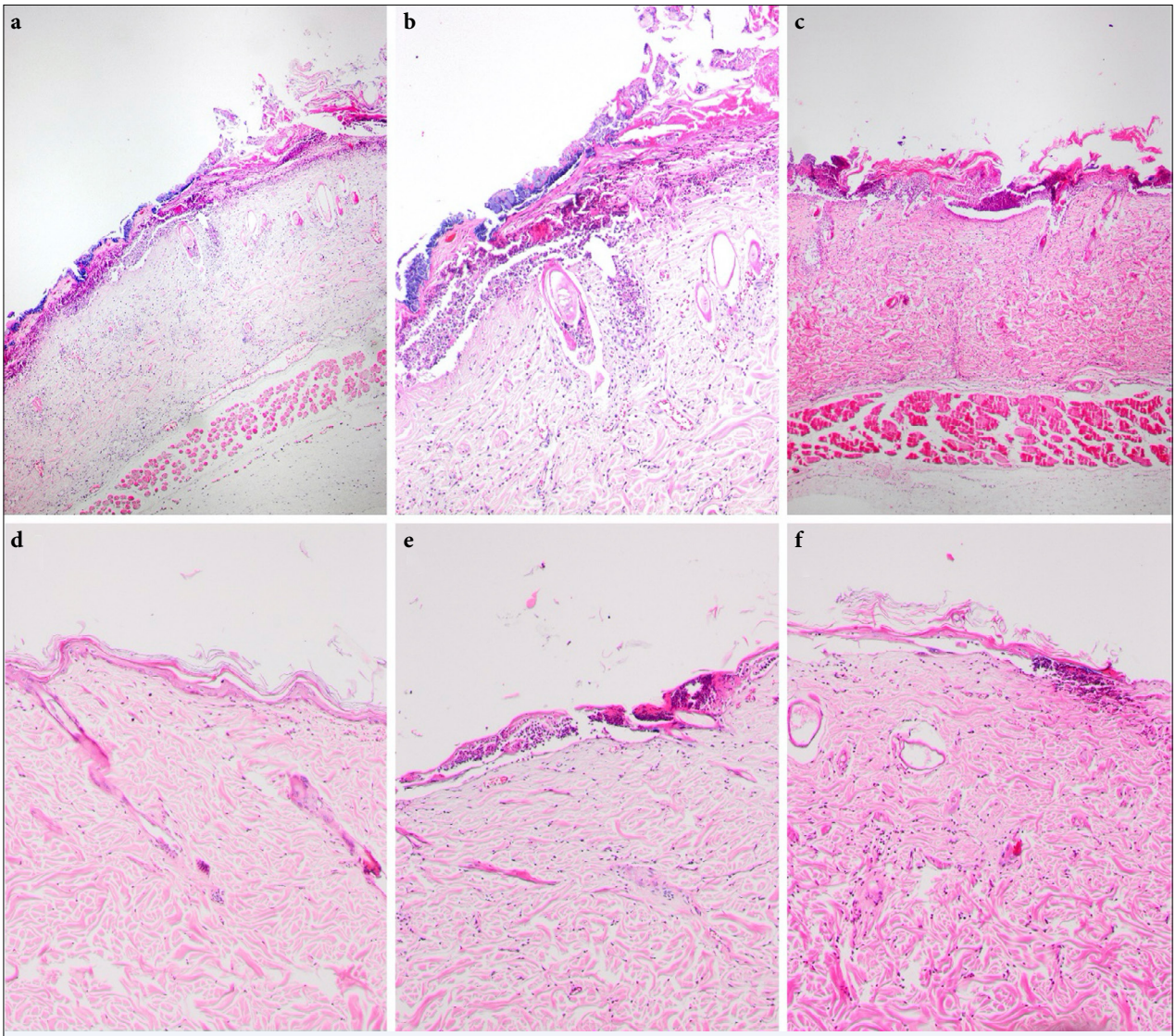

Fig. 4. (a-c) Representative rat skin from *Hypericum Perforatum* oil-treated group, scarring and diffuse-severe inflammation, (a, c) 40x field (b) 100x field. (d) Representative rat skin from the control group, no inflammation, 100x field. (e, f) Another representative image form control group with focal-moderate inflammation, 100x field.

inflammation in the histopathological examination with HP oil. HP oil is an over-the-counter treatment method that is frequently used by patients. It remains unaddressed during the standard medical history assessment, with individuals opting not to provide this information to their attending healthcare provider. Therefore, the use of HP oil should be questioned before RT and should not routinely be recommended to patients.

In a single-arm prospective observational study in which HP oil was used to treat RD, an ointment containing HP oil and neem oil was started when bright erythema, moderate edema, or patchy moist desquamation was observed in patients who underwent chemoradiotherapy (CRT) in the head and neck (H&N) region, and this treatment was found to be safe and effective. [12,13] The contradictory outcomes obtained in our study and the H&N studies investigating the same question might be attributed to two main factors. First, in Narayanan et al.[23],'s study, HP oil was used as an ointment together with neem oil. Consequently, the concentration of HP oil in the ointment is diminished, resulting in a corresponding decrease in the concentration of hypericin. Neem oil is an agent whose antibacterial properties have been well demonstrated. Neem oil might have compensated for the increased RD effect of HP oil. The second main difference is the prophylactic

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initiation of HP oil in our study. Franco et al.[13] started to apply the remedy containing HP oil after grade 2 dermatitis was observed. The presence of inconsistent findings could possibly be attributed to variations in the methodologies employed across different research projects. In order to obtain a more definitive understanding of the matter at hand, it is imperative to conduct future prospective clinical investigations specifically focusing on the application of an ointment including only HP oil. The possibility that HP wort may cause increased radiosensitivity first emerged in the literature with a case report published in 2006. Putnik et al.[21] reported a case of recall dermatitis in a patient who had undergone adjuvant RT for laryngeal cancer. The dermatitis occurred one year after RT and was associated with the administration of hypericin. Eichkorn et al.[20] reported that HP oil produced significant photon radiosensitivity and that severe folliculitis capitis was found on the scalp of the patient who underwent whole-brain RT. The existing literature consists solely of two case reports that demonstrate radiosensitivity with HP oil. Nevertheless, research was conducted to analyze the levels of plasma hyperforin and hypericin during hospitalization, revealing positive results in 11.3% of patients.[24] The fact that this herbal remedy, which is frequently self-prescribed by patients, has an enhanced photosensitizing impact that is not addressed in the literature might be for a number of various reasons. Due to the frequent utilization of this wort as a therapeutic agent for skin lesions, it is plausible that the augmented radiosensitivity effects may go unnoticed by patients or remain undisclosed to their healthcare providers. Additionally, it is conceivable that a threshold exists for the radiation-sensitizing effect of products containing HP.

Unlike the photon radiosensitizing effect of HP, its photosensitizing effects have been frequently studied in the literature. The first publication showing increased photosensitivity with the topical application of HP wort belongs to Schempp et al.[18] In this study, the effects of hypericum oil (hypericin 110 mg/mL) and hypericum ointment (hypericin 30 mg/mL) on skin sensitivity to solar radiation were examined, and hypericum oil has been shown to cause an increased erythema index. Increased photosensitivity with HP oil strengthens the idea that hypericin has an effect on a certain threshold value, as was previously stated. The photosensitivity-enhancing effects of HP have also been demonstrated in preclinical studies of transitional cell bladder cancer.[15,25] Although HP's collaboration with photodynamic therapy has also been demonstrated in anaplastic thyroid and melanoma cells, in one particular clinical study, no photosensitizing effect was demonstrated when HP extract was applied to skin lesions.[16,17,26] The contradictory results in the studies and the heterogeneity of the literature cause confusion. Therefore, in studies where HP wort will be used, especially in topical applications, the poor penetration rate due to the high molecular weight and high melting point of hypericin and the possible photo/radiosensitizing cut-off value should be kept in mind, and studies should be designed accordingly.[18]

Limitations of the Study

Our study has several limitations. The most significant is the small sample size and the loss of two animals in the HP oil-treated group during the experiments. Additionally, our study focused exclusively on HP oil, so different outcomes might be observed with other HP extracts.

CONCLUSION

To conclude, our findings indicate that the use of HP oil simultaneously with RT resulted in an elevation of the post-radiation skin damage score in rats over the follow-up period. Histopathological analyses revealed that this combination led to an augmentation in both the occurrence and intensity of scar formation and inflammation. Our data suggest that HP oil worsens RD when administered concurrently with RT. In routine clinical practice, it is advisable to inquire about patients' use of HP supplements, and caution should be exercised while recommending it.

Ethics Committee Approval: The study was approved by the Kobay Local Ethics Committee (no: 428, date: 11/11/2019).

Authorship contributions: Concept – M.T.Y., F.Y.Y., S.Y.S., O.K., M.C., F.Z., G.Y.; Design – M.T.Y., F.Y.Y., S.Y.S., O.K., M.C., F.Z., G.Y.; Supervision – S.Y.S., O.K., M.C., F.Z., G.Y.; Funding – S.Y.S., M.C., F.Z., G.Y.; Materials – M.T.Y., F.Y.Y., S.Y.S., O.K., M.C., F.Z., G.Y.; Data collection and/or processing – M.T.Y., F.Y.Y., S.Y.S., O.K., M.C., F.Z., G.Y.; Data analysis and/or interpretation – M.T.Y., F.Y.Y., S.Y.S., O.K., M.C., F.Z., G.Y.; Literature search – M.T.Y., F.Y.Y., G.Y.; Writing – M.T.Y., F.Y.Y., G.Y.; Critical review – M.T.Y., F.Y.Y., S.Y.S., O.K., M.C., F.Z., G.Y.

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