PHARMACOLOGY AND PHARMACY

OBTAINING EMULSIONS OF FURANOCOUMARINS FROM SOSNOWSKY'S HOGWEED AND *IN VITRO* ASSESSMENT OF THEIR PHOTOTOXIC EFFECT

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ABSTRACT

Background. *Furanocoumarin-based drugs are used for photochemotherapy of various diseases. Sosnovsky's hogweed can be an available source of furanocoumarins for the development of drugs.*

The aim of the study. *To obtain stable emulsions containing furanocoumarins from Sosnowski's hogweed and to evaluate their photocytotoxicity.*

Materials and methods. *To obtain the emulsions, furanocoumarins were extracted with chloroform from the sap of the aerial part of the Sosnowski's hogweed. The chloroform extract was clarified by silica gel gradient column chromatography. The extractive fraction containing furanocoumarins was analyzed by high performance liquid chromatography with ultraviolet (UV) detection.*

An extract with a high content of 8-methoxypsoralen was used to prepare two types of emulsions. The extract was dissolved in peach oil and emulsified in water with tween-80 (emulsion No. 1) and in an aqueous glycerin solution with lecithin (emulsion No. 2).

The emulsions were tested for dark and photo-induced toxicity for human lung fibroblasts. The dose of UV radiation for the photoactivation of furanocoumarins was 9 J/cm^2. A solution of chlorine e6 was used as a comparison photosensitizer.

Results. *The obtained emulsions contained 1 mg/ml 8-methoxypsoralen. Both emulsions were homogeneous at macro- and microscopic visualization, remained stable when stored under various temperature conditions for 14 days. Emulsion No. 2 did not show dark toxicity and caused a statistically significant inhibition of cell viability under UV irradiation at a concentration of 12.5–31.3 μg/mL. Emulsion No. 1 had a toxic effect on cells regardless of UV irradiation due to the content of tween-80. According to fluorescent microscopy, the phototoxic effect of emulsion No. 2 was manifested mainly due to apoptosis, in contrast to the effect of chlorine e6, in which there were more pronounced signs of cell necrosis.*

Conclusion. *The developed experimental emulsions of furanocoumarins from Sosnovski's hogweed are an example of promising medicinal photosensitizers of plant origin for phototherapy of various dermatological and oncological diseases.*

Key words: *emulsion, furanocoumarins, Sosnowski's hogweed, UV radiation, tween-80, lecithin, apoptosis*

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ПОЛУЧЕНИЕ ЭМУЛЬСИОННЫХ ФОРМ ФУРАНОКУМАРИНОВ БОРЩЕВИКА СОСНОВСКОГО И ОЦЕНКА ИХ ФОТОТОКСИЧЕСКОГО ДЕЙСТВИЯ *IN VITRO*

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РЕЗЮМЕ

Обоснование. *Для фотохимиотерапии различных заболеваний используются средства на основе фуранокумаринов. Доступным источником фуранокумаринов для создания лекарственных препаратов может быть борщевик Сосновского.*

Цель исследования. *Получить стабильные эмульсии, содержащие фуранокумарины, из борщевика Сосновского и оценить их фотоцитотоксичность.*

Материалы и методы. *Фуранокумарины для получения эмульсий экстрагировали хлороформом из сока надземной части борщевика Сосновского. Хлороформный экстракт очищали с помощью градиентной колоночной хроматографии на силикагеле. Экстрактивную фракцию, содержащую фуранокумарины, анализировали с помощью высокоэффективной жидкостной хроматографии с ультрафиолетовым (УФ) детектированием.*

Экстракт с высоким содержанием 8-метоксипсоралена использовали для получения двух видов эмульсий. Экстракт растворяли в персиковом масле и эмульгировали в воде твином-80 (эмульсия № 1) и в водно-глицериновом растворе лецитином (эмульсия № 2).

Эмульсии тестировали на темновую и фотоиндуцированную токсичность для фибробластов лёгких человека. Доза УФ-излучения для фотоактивации фуранокумаринов составила 9 Дж/см^2. В качестве фотосенсибилизатора сравнения использовали раствор хлорина е6.

Результаты. *Полученные эмульсии содержали 1 мг/мл 8-метоксипсоралена. Обе эмульсии были гомогенными при макро- и микроскопической визуализации, сохраняли стабильность при хранении в различных температурных условиях в течение 14 дней. Эмульсия № 2 не проявляла темновой токсичности и вызывала статистически значимое угнетение жизнеспособности клеток при УФ-облучении и концентрации 12,5–31,3 мкг/мл. Эмульсия № 1 оказывала токсическое действие на клетки независимо от УФ-облучения из-за содержания в составе твина-80. По данным флуоресцентной микроскопии, фототоксическое действие эмульсии № 2 проявлялось главным образом за счёт апоптоза, в отличие от действия хлорина е6, при котором имелись более выраженные признаки некроза клеток.*

Заключение. *Разработанные экспериментальные эмульсионные формы фуранокумаринов борщевика Сосновского являют собой пример перспективных лекарственных фотосенсибилизаторов растительного происхождения для фототерапии различных заболеваний в сфере дерматологии и онкологии.*

Ключевые слова*: эмульсия, фуранокумарины, борщевик Сосновского, УФ-излучение, твин-80, лецитин, апоптоз*

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OBJECTIVES

One of the substances widely used in medicine for photochemotherapy are furanocoumarins, among which psoralen derivatives (5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP), etc.) have clinically proven effect [1]. Sosnowsky's hogweed stands out among the plants containing high concentrations of psoralen and its derivatives [2]. In some regions of the globe, including Russia, Sosnowsky's hogweed, as well as other species of giant hogweeds, is recognized as a dangerous phytoinvasive species and is widely eradicated [3]. As Sosnowsky's hogweed is widespread and rapidly growing phytomass, its plant raw material is very accessible for harvesting and pharmaceutical use.

Currently, the plant *Ammi majus* is the source of furanocoumarins for the production of Ammifurin, photosensitizing drug. Its fruits contain about 2 % furanocoumarins. Ammi preparations are used as antipsoriatic agents for PUVA (psoralen and ultraviolet A) therapy [4].

Ammi majus is inferior to Sosnowsky's hogweed in phytomass for the production of furanocoumarins. Ammi furanocoumarins are localized predominantly in the fruits of the plant and are mixed with a large number of hydrophobic related substances [4]. Ammi majus can be found wild only in warm climatic conditions [5, 6] and in other regions it requires special cultivation conditions.

Furanocoumarins are very lipophilic substances, but they must be in an aqueous environment to realize their photobiological potential [7]. In various studies, furanocoumarins are tested as alcoholic solutions or dimethyl sulfoxide (DMSO) solutions [8, 9], where furanocoumarins remain stable at high concentrations (more than 1 mg/ml) provided at least 80 % DMSO is present in the solvent. The problem of solubility of furanocoumarins in water can be solved by creating an emulsion form [10]. Obtaining an emulsion form of furanocoumarins may alter or disappear the native photosensitizing effect induced by the plant itself [11]. Until now, furanocoumarin emulsions based on medicinal plant raw materials have not been developed and used. In this regard, it becomes relevant to control the specific activity of active substances, in our case furanocoumarins.

An effective dosage form of furanocoumarins with available raw materials for its manufacture can be widely used as an agent for PUVA therapy or as an antineoplastic agent against skin neoplasia: for example, microemulsion for transcutaneous delivery of furanocoumarins containing various emulsifiers (isopropyl myristate, tween-80, span-80, octanediol) [10]. Microemulsions of 8-methoxypsoralen are intended for PUVA therapy as topical photosensitizers. However, the correlation between dark and photoinduced toxicity is unfairly little considered in such studies.

There is a view that a simple photosensitizer emulsion is not suitable for emulsion droplets without polymer coatings, and the photosensitizing effect of simple and polymer-associated emulsions has not been compared [11].

The use of nanoemulsions of furanocoumarins, where essential oil is used as the oil phase, has been proposed for local administration. It has been found that despite the finely dispersed composition (about 30 nm) of the resulting emulsion, it is also well retained in the skin [12]. However, we cannot, for example, talk about the parenteral administration in case of such emulsions.

Researchers offering emulsion formulations for skin application have a fairly wide choice of solvents, as furanocoumarins can dissolve in many essential and fatty oils. However, if the photosensitizer is to be administered parenterally, the range of oils available for use is sharply narrowed. The need to administer the photosensitizer intravascularly may be motivated by a more significant effect, as opposed to superficial application, which has been repeatedly shown by various researchers [13, 14]. Parenteral formulation of the photosensitizer is also necessary for intratumoral administration during therapy of unresectable tumors [15, 16] or delivery of activating radiation via optical fiber into the tumor tissue [17].

The aim of this study was to obtain stable emulsions containing furanocoumarins isolated from Sosnowsky's hogweed and to evaluate their photocytotoxicity.

MATERIALS AND METHODS

The source of plant raw material was the aerial part of *Heracleum sosnowskyi* Manden. The plant was defined according to *The Giant Hogweed Best Practice Manual. Guidelines for the Management and Control of an Invasive Weed in Europe* (2005). The furanocoumarins fraction was extracted from the sap of the aerial part. The extraction, purification and high-performance liquid chromatography (HPLC) process was carried out according to the previously described methodology [18], the scheme of which is shown in Figure 1.

Two emulsions with different emulsifying agents were prepared from the extractive fraction containing furanocoumarins. For the manufacturing of emulsions, the dried extract containing 100 mg of 8-MOP was dissolved on heating in 10 ml of peach oil (Mirrolla, Russia). Further work was carried out with the heated oil extract solution for emulsion manufacturing.

An aqueous 2.5 % solution of polysorbate 80 (Tween-80) (Sigma-Aldrich, USA) was used as a stabilizer for the first method of furanocoumarins emulsion (emulsion No. 1) manufacturing. Under vigorous stirring and constant temperature of 25 °C, 1 ml (0.916 g) of an oil solution of furanocoumarins of Sosnowsky's hogweed with a concentration of 8-MOP 6.125 mg/ml was added dropwise to 5 ml of polysorbate solution. The prepared mixture was stirred for 10 minutes and then exposed to ultrasound (50 W) for 10 minutes. A total of 3 repetitive emulsification cycles were performed. The quality of the prepared emulsion was controlled by light microscopy in three stages: 1) immediately after preparation;

2) after storage in a refrigerator for 96 h at 4 °C; 3) after storage at 20–22 °C for 14 days.

The second variant of furanocoumarins emulsion (emulsion No. 2). The aqueous phase was made by adding 0.1315 g of glycerol (Sigma-Aldrich, USA) to 5 ml of deionized water; the mixture was stirred on a magnetic stirrer at 70–75 °C. At the same time, an oil phase was prepared from 0.1 g of lecithin (phosphatidylcholine, EPCS 10 8018-1/130, Lipoid, Germany) and 0.916 g of an oil solution of hogweed furanocoumarins obtained according to the method described above. The mixture was stirred at 90 °C until a homogeneous consistency is obtained. The oil phase was slowly added to the aqueous phase at a temperature of 70–75 °C while stirring vigorously. The resulting emulsion was stirred at 70–75 °C for 10 minutes. The mixture was then homogenized using ultrasound (200 W – 1 min; 2 s/2 s cycle) until emulsion globules were formed.

The study of emulsion cytotoxicity was carried out on cell culture of human lung fibroblasts (HLF) in the National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of Russia. During the exponential growth phase, cells were dispersed into a 96-well plate at a concentration of 5,000 cells/well and incubated for 24 h under standard conditions on DMEM medium supplemented with 10 % FBS (fetal bovine serum) and antibiotics (penicillin-streptomycin) at 5 % CO_2 and 37 °C.

Phototoxic reactions of furanocoumarins were induced using an ultraviolet (UV) lamp (365 nm, 40 W; Camelion LH26-FS/BLB/E27, China), which was placed at a fixed distance of 20 cm from the target cells to obtain a constant radiation intensity of 35 mW/cm^2. Radiation intensity was measured using a ThorLabs PM100D radiometer (Germany). The photocytotoxic effects of the emulsions were determined at concentrations of 8-MOP in the well of the plate: 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2 and 1 μg/ml. Cells were irradiated with UV light four hours [19] after emulsions were applied.

Chlorin e6, a photosensitizer from the group of porphyrins, was used to control photodynamic stress. Chlorin e6 solution (50 μg/ml) was prepared in DMSO (50 mg/ml) and stored in a dark place at 4 °C. For the induction of photodynamic action with chlorin e6, concentrations in the well of the plate were used: 6.3–0.05 μg/ml [20, 21]. The cells were irradiated with 660 nm LED lamp irradiation.

The radiation dose was selected empirically based on the results of a control experiment without application of the test agents. 1 hours after irradiation, cells were trypsinized and transferred to a 96-well plate with medium. After 24 hours of incubation, cell viability was assessed using the MTT assay [22]. Tween-80 and lecithin were administered separately to assess their contribution to cytotoxic effects. The phototoxic effect of each photosensitizer was monitored in relation to cells

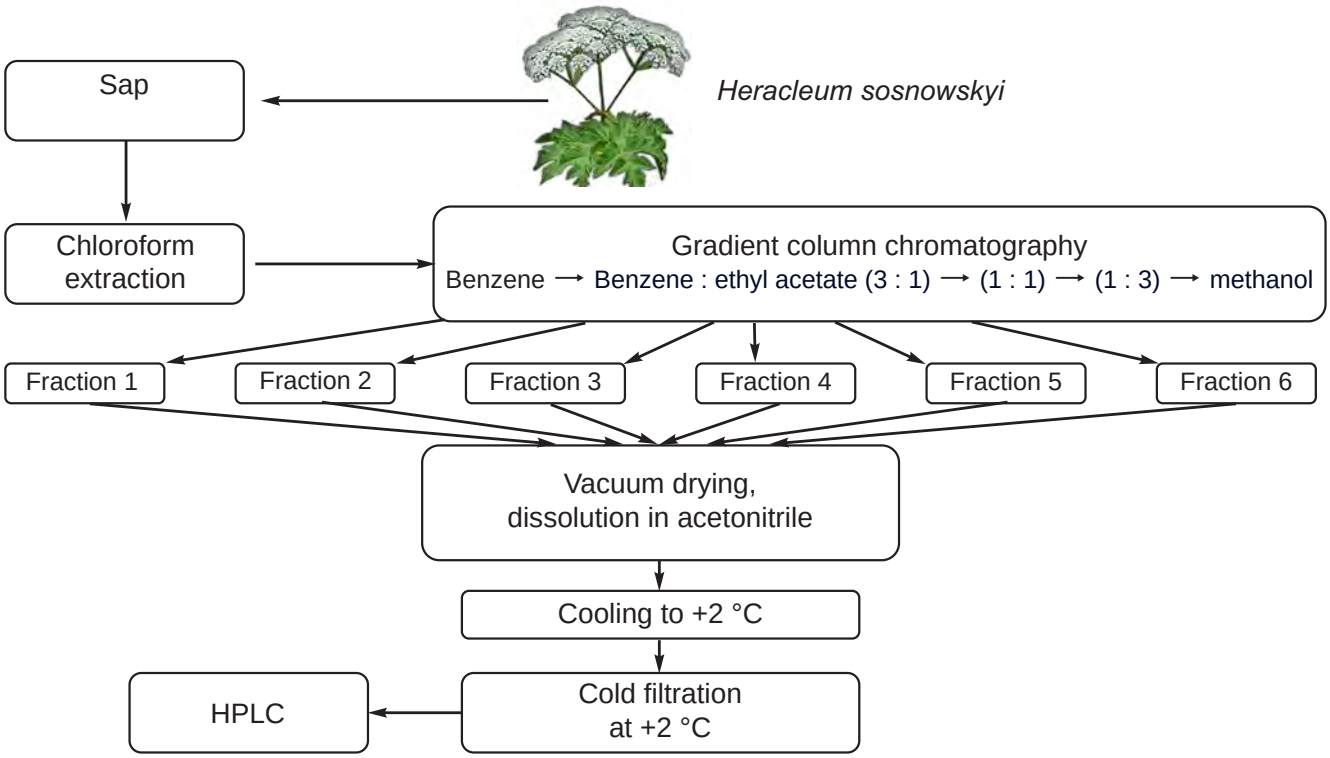

FIG. 1.

Scheme for isolating the furanocoumarin fraction from the Sosnowsky's hogweed sap. High-efficiency liquid chromatography of extractive fractions was carried out in isocratic mode (chromatography system by Gilson (France); Kromasil C18 column 4.5 mm × 5 μm × 250 mm). Mobile phase: water/acetonitrile (1:1). Flow rate 0.8 ml/min. Detection at wavelength 250 nm. Software "Millichrome" (Russia). Analytical standards: 5-methoxypsoralen, 8-methoxypsoralen (Sigma-Aldrich, USA)

that were placed in a dark place after administration of the test substances. Cell viability in all experimental groups was evaluated relative to a control series of wells (negative control) in which no test component was placed and no irradiation was performed.

After 24 hours of incubation, the medium was replaced with 5 % MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl). MTT was reduced to formazan crystals, which was evaluated using an inverted microscope (Micromed, Russia). The medium was then removed and 150 µl of DMSO was added and stirred for 20 min at 37 °C. Optical density was measured on Varioskan Lux Reader (Thermo Fisher Scientific, USA) at a wavelength of 570 nm versus 650 nm reference wavelength. Optical density (OD) results were displayed in % OD (sample) / OD (control).

Data are presented as mean ± standard error of mean (SEM) for > 3 independent experiments or half maximal inhibitory concentration (IC_{50}) values and their 95 % confidence intervals obtained by nonlinear regression. Differences between experimental groups were compared by statistical data processing using Student's t-test and ANOVA test. The critical level of statistical significance of the differences was 5 % ($p < 0.05$). All statistical analyses were performed using SPSS Statistics 10.0 software (IBM Corp., USA).

RESULTS

A fraction with high content of furanocoumarins (8- and 5-methoxypsoralen) was found using HPLC of the extract. The appearance of the chromatograms is shown in Figure 2.

The retention time of the substances of the Sosnowsky's hogweed extract corresponded to the retention time of the analytical standards 8- and 5-MOP. According to the calibration curve (peak area versus concentration) constructed using a solution of 5- and 8-MOP in acetonitrile, the concentration of 8-MOP in the original sap was 506 mg/l, and that of 5-MOP was 23 mg/l.

Based on the predominance of 8-MOP in the sap of Sosnowsky's hogweed, this furanocoumarin was taken as the dosage active substance in the emulsions.

When the concentration of 8-MOP in peach oil was 6.125 mg/ml at room temperature (20 °C), no needle crystals of furanocoumarins were formed and a stable oil solution was obtained. However, when the solution was placed in a refrigerator at 4 °C, needle crystal precipitation occurred. The concentration in the 8-MOP oil solution that did not result in crystals at 20 °C and 4 °C was 3 mg/ml. The experimentally obtained solubility limit of extractive furanocoumarins in oil is similar to that obtained by B. Baroli et al. for chemical substances [10].

The quantities of emulsion ingredients are listed in Table 1. Both emulsions contained 8-MOP at a concentration of 1 mg/ml.

The obtained emulsions were homogeneous visually and under microscopy. The emulsion samples remained stable during a storage period of 14 days or more with the temperature range of 4–20 °C.

UV radiation had a statistically significant inhibitory effect on HLF cell development at a dose of 22 J/cm^2. Thus, the ultimate safe dose of this radiation was 18 J/cm^2 (Fig. 3a). However, this dose had a large error in the mean, and we adopted a UV dose of 9 J/cm^2 as an indifferent dose for this cell type. LED wavelength radiation (660 nm) did not cause a statistically significant decrease in cell viability up to a dose of 30 J/cm^2 (Fig. 3a) and was used at this power to photoactivate chlorin e6.

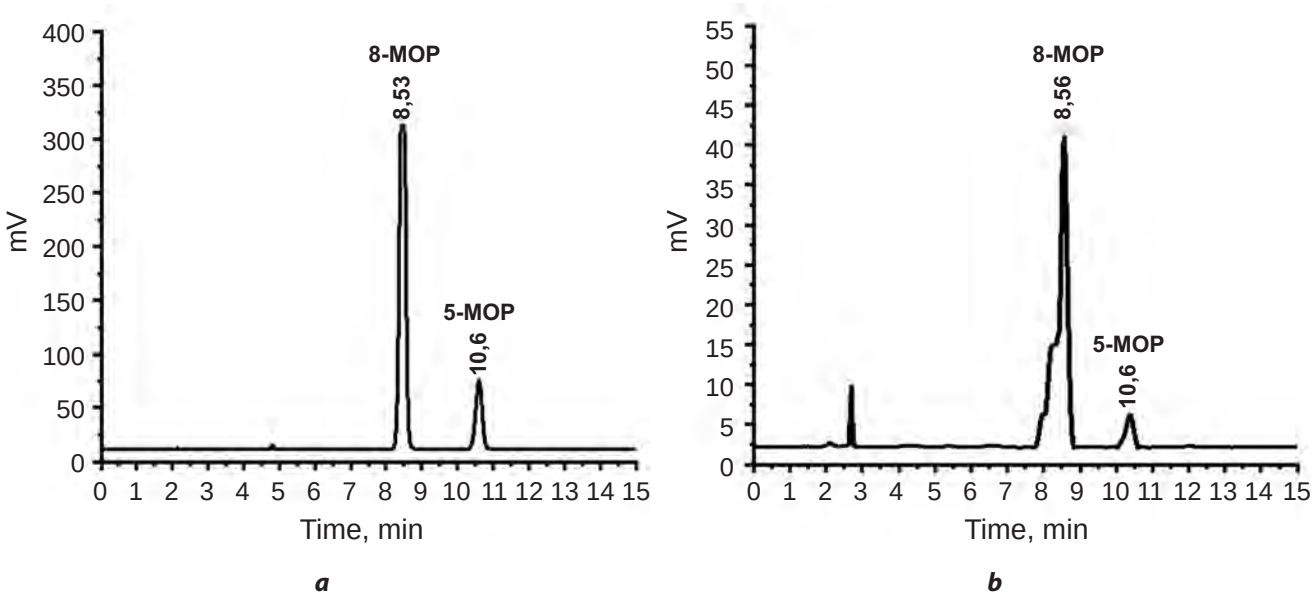

FIG. 2.

*Chromatograms of a solution of furanocoumarins (8- and 5-methoxypsoralen) in acetonitrile (**a**) and of the extract of Sosnowsky's hogweed sap containing high concentrations of the same furanocoumarins (**b**)*

TABLE 1

COMPOSITION OF OBTAINED EMULSIONS OF FURANOCOUMARIN FROM SOSNOWSKY'S HOGWEED

Composition of emulsion sample No. 1 (w/w %)			
Water	Peach oil	8-MOP	Tween-80
82.7	15.15	0.1	2.05
Composition of emulsion sample No. 2 (w/w %)			
Water	Glycerin	Peach oil	8-MOP	Lecithin
81.3	2.12	14.89	0.1	1.63

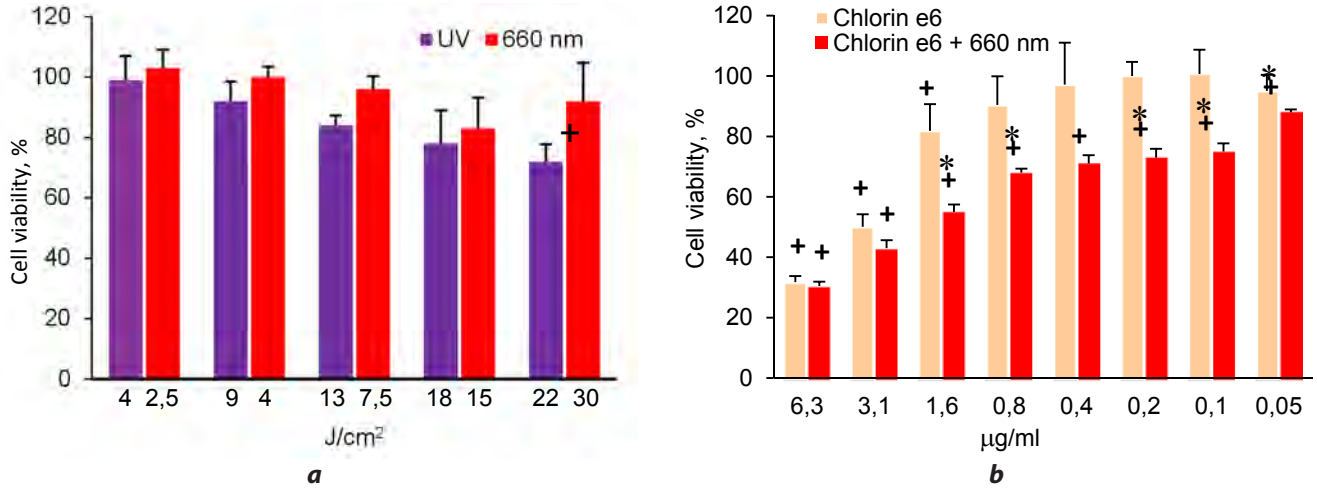

FIG. 3.

*Change in viability of HLF cells under different doses of UV (365 nm) and red (660 nm) radiation (**a**); photodynamic effect of chlorin e6 on HLF cells (**b**): + – statistically significant differences with values of the control group, without application of test substances and irradiation; * – statistically significant differences with values without irradiation at the same concentration of chlorin e6*

MTT assay in the group using the photodynamic photosensitizer chlorin e6 showed statistically significant changes in HLF cell viability due to the photosensitizing effect. At concentrations of 1.6–6.3 µg/ml, chlorin e6 had a significant toxicity against HLF cells, as at these concentrations cell viability was statistically significantly reduced relative to the negative control group in both cultivation regimes (Fig. 3b). Starting from chlorin concentration e6 1.6 µg/ml, the photosensitizing effect of chlorin began to appear, as in this case cell viability was statistically significantly lower ($p < 0.01$) than in the dark regime (Fig. 3b). At lower concentrations of chlorin e6, its phototoxicity was still present at all dilutions (0.05–0.8 µg/ml), as evidenced by the low cell viability (at 68–75 %), which had statistically significant differences from that in the group of cells that received chlorin at the same concentrations but were not exposed to LED irradiation (Fig. 3b). At a chlorin e6 concentration of 0.4 µg/ml and irradiation, no statistically significant differences with the dark regime were noted. However, relative to intact cells, lower viability was noted at $p < 0.001$ (Fig. 3b).

Figure 4a shows that emulsion No. 1 has a cytotoxic effect on cells at fairly low concentrations. It is shown that there is no significant difference in cell survival without and after UV irradiation. Up to a concentration of 2 µg/ml in 8-MOP medium, emulsion No. 1 shows statistically significant cytotoxicity. However, if the intrinsic toxicity of tween-80 is considered (Fig. 4b), it can be seen that it repeats in general the toxicity of emulsion No.1.

Emulsion No. 2 without UV irradiation had no toxic effect on cells. In contrast to emulsion No. 1, lecithin was used as an emulsifier in emulsion No. 2, which did not show its own cytotoxicity at the concentrations studied. Lecithin, examined in isolation from other emulsion components, did not cause statistically significant changes in HLF culture viability under both dark and UV irradiation conditions (Fig. 4b).

When emulsion No. 2 is used in combination with UV, a clear phototoxic effect can be observed against cells at 8-MOP concentrations of 125, 62.5 and 31.3 μg/ml. At these concentrations, emulsion No. 2 reduced cell viability to 38 ± 9, 25 ± 6 and 41 ± 3 %, respectively, versus 86 ± 6 % ($p < 0.01$), 110 ± 1 % ($p < 0.001$) and 87 ± 3 % ($p < 0.001$) at the same concentrations but under dark conditions (Fig. 4b).

Relative to intact cells of the negative control, cells that received emulsion No. 2 in photomode also had statistically significantly lower viability values. Thus, at 8-MOP concentrations of 31.3, 62.5 and 125 μg/ml and UV irradiation, cell viability was statistically significantly lower than in the control at the statistical significance level of 0.017, 0.0048 and 0.018, respectively (Fig. 4a).

At lower concentrations (1–15.6 μg/ml), emulsion No. 2 did not inhibit cell viability under both dark and UV conditions. Therefore, it can be concluded that the concentration of 8-MOP 31.3 μg/ml for this type of cells appeared to be the limit for realization of phototoxic action of emulsion No. 2.

Fluorescence microscopy showed that in a large percentage of cases, cells lost viability due to apoptosis (Fig. 5). Apoptotic cells at early and late stages were detected in all test groups. Without cell culture intervention, the percentage of cells in apoptosis was 7 ± 3 %. After UV irradiation (9 J/cm^2) it increased to 15 ± 3.5 %, which was not statistically significant. A distinctive feature of cells with signs of apoptosis in the group with emulsion No. 2 and chlorin e6 after photoactivation was the presence of vesicles on the cell membrane. Almost all cells in these groups had this trait after irradiation (Fig. 5). The highest number of apoptotic cells could be noted in the group with emulsion No. 2 after UV irradiation, where 84 ± 6 % of all cells showed signs of apoptosis. Necrotic cells with karyorrhexis and monotonous red and brown cytoplasm were found most extensively in the groups with emulsion No. 1 and tween-80 in 25 % and 28 % of cases, respectively.

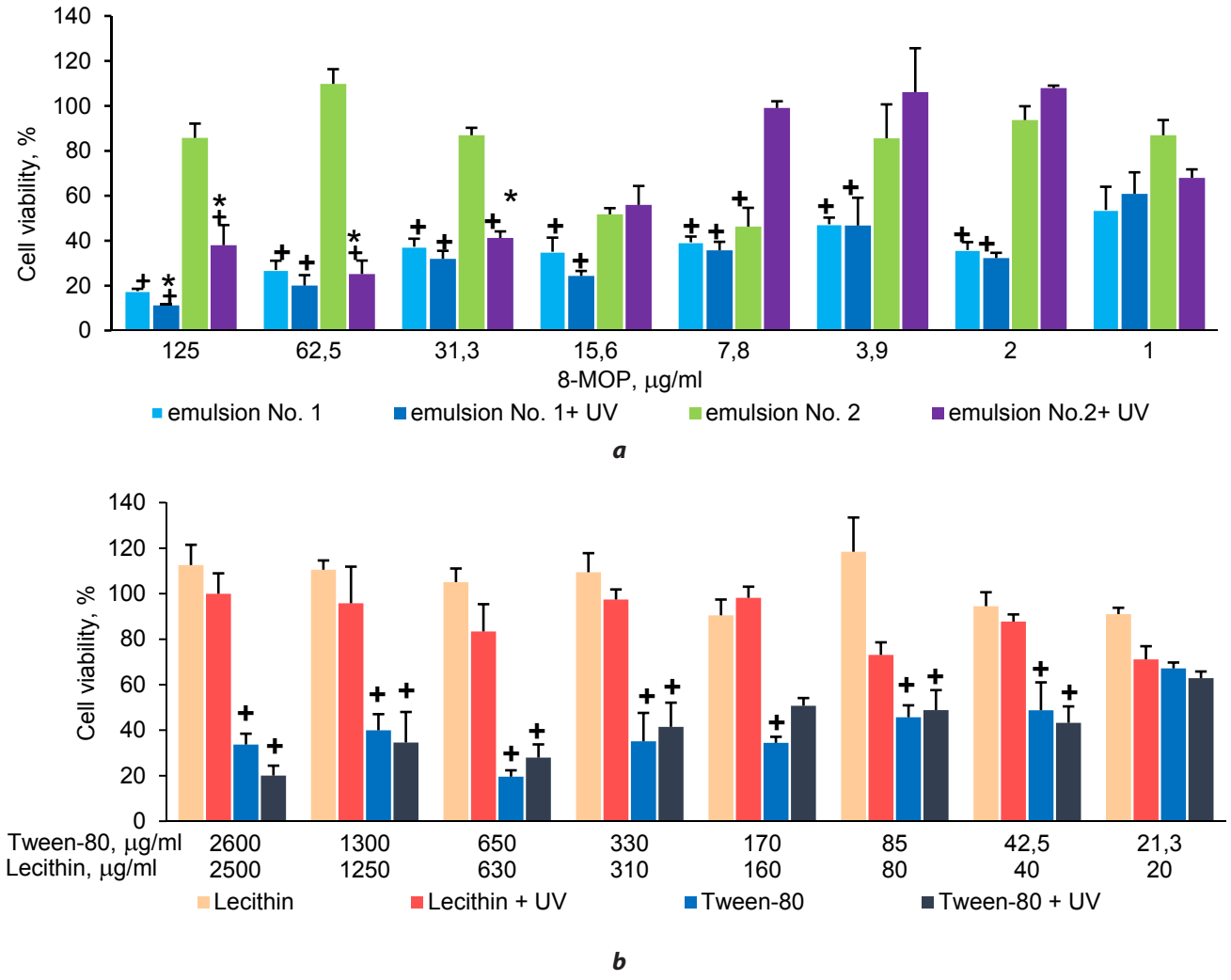

FIG. 4.

*Viability of HLF cells according to the MTT test under the influence of emulsion furanocoumarins from Sosnowsky's hogweed (**a**) and of used emulsifiers (**b**) (dark and photo-induced toxicity): + – statistically significant differences from the values in the control group, without the test substances introduction and irradiation; * – statistically significant differences from the values without UV irradiation and at the same concentration of test substances*

TABLE 2

RATIO OF DARK AND PHOTOINDUCED CONCENTRATION (MG/ML) OF HALF MAXIMAL INHIBITION ($IC_{50\ DARK}/IC_{50\ UV}/660$) FOR 8-MOP IN EMULSIONS, EMULSIFIERS AND CHLORIN E6

Emulsion No. 1 (8-MOP)	Tween-80	Emulsion No. 2 (8-MOP)	Lecithin	Chlorin e6
1.2/10.5	51/69.5	–/36.8	–/–	4/2

At the same time, the number of cells with signs of apoptosis and necrosis did not statistically significantly increase under UV irradiation (Fig. 5). The increase in cells showing signs of apoptosis and necrosis when exposed to UV irradiation on cells treated with emulsion No. 2 was similar to that of chlorin e6, where after red light irradiation an average of 55 ± 2 % of cells were in a state of apoptosis and 14 ± 1 % were in a state of necrosis. In the group treated with lecithin at a concentration of 2.5 mg/ml, vesicles on the cell membrane were extremely rare, and the morphological pattern of this group did not change after UV irradiation.

Injection of lecithin into the medium with cells resulted in 24 ± 2 % of cells showing signs of apoptosis (Fig. 5). However, UV irradiation did little to change cell morphology upon lecithin application with 40 ± 8% of cells in a state of apoptosis.

The concentration of half-maximal inhibition of 8-MOP in emulsion No. 1 did not decrease but increased upon UV irradiation of cells (Table 2), which was apparently due to the prevailing toxicity of tween-80 for this emulsion, which prevented the detection of the photosensitizing effect of 8-MOP *in vitro*.

Emulsion No. 2 was responsive to UV irradiation and markedly reduced cell viability. In the absence of UV, emulsion No. 2 was found to be non-toxic to cells and IC_{50} in a dark place was not reached for this emulsion (Table 2).

DISCUSSION

Therefore, emulsion No. 2 has a photocytotoxic effect due to the furanocoumarins of Sosnowky's hogweed contained in it and is not toxic to human cells in the absence of UV radiation. Emulsion No. 1 has its own cytotoxicity due to its tween-80 content. Tween-80 has previously been shown to have membranotoxic properties [23]. However, the toxicity of tween-80 does not negate the presence of photoinduced toxicity in emulsion No. 1, as the *in vitro* toxicity of tween-80 may override it. The development of a stable emulsion dosage form in our study was aimed at the prospective use of furanocoumarins *in vivo* to create an affordable and easy to manufacture dosage form for photochemotherapy [24]. The emulsion form studied overcomes the difficulties associated with the hydrophobicity of furanocoumarin molecules and effectively stabilizes them as a liquid heterogeneous system [25]. Lecithin used to create emulsion No. 2 showed itself as a photo-independent component, which is consistent with the literature [26], and therefore did not interfere with the phototoxic effect of furanocoumarins.

Despite the fact that Sosnowsky's hogweed is a dangerous invasive plant growing in many regions of the world. Nevertheless, its physiological features and chemical composition make it a unique and accessible source of raw materials for the production of highly active photosensitizers, which may become an alternative to phthalocyanines against various diseases, including neoplasms, requiring the use of photoactive materials [16]. As our study showed, furanocoumarins of Sosnowsky's hogweed have a phototoxic effect on isolated cells, unlike the standard photosensitizer chlorin e6 – mainly due to the activation of apoptosis (Fig. 5). A characteristic morphological sign of cell apoptosis under the influence of furanocoumarins of Sosnowsky's hogweed was the presence of vesicles on the cytoplasmic membrane. This probably complements the mechanism of photocytotoxicity of the hogweed furanocoumarins and suggests their membranotoxic action [27]. The same mechanism of phototoxicity is characteristic of photosensitizers, porphyrin derivatives, which require oxygen and the activator, light, for activation [28]. However, the mechanism of photosensitizing action of furanocoumarins is related to the formation of A-covalent monoadducts and interchain cross-links in DNA between pyrimidine bases under UV radiation [29]. This mechanism is not the classical photodynamic mechanism characteristic of chlorin e6 and other porphyrin-type photosensitizers.

CONCLUSION

The developed experimental emulsions of furanocoumarins from Sosnowsky's hogweed are an example of promising medicinal photosensitizers of plant origin for phototherapy of various dermatological and oncological diseases.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

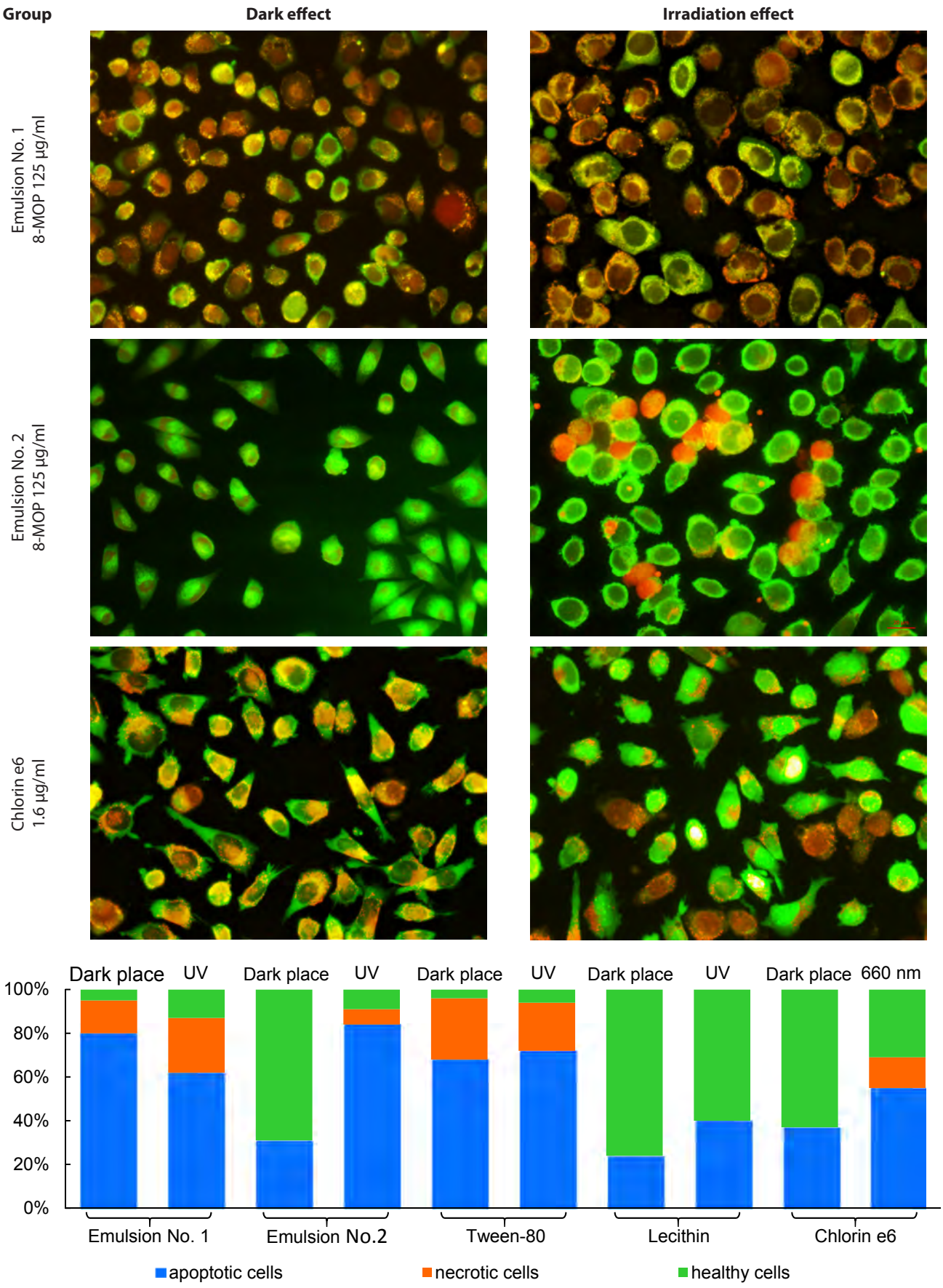

FIG. 5.

Morphology and quantitative evaluation of HLF cells with signs of apoptosis and necrosis under the influence of photosensitizers in the dark and upon photoactivation (explanation in the text)

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