

**ECB
2021**

The 1st International Electronic Conference on Biomedicine

01-26 JUNE 2021 | ONLINE



Photosensitizer chlorophyllin in the treatment of oncopathologies

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Abstract: In this article we report the antitumor activity of the derivative chlorophyllin with Cu (CuChlNa) and Chlorin e6 (Ce6). Ehrlich' s ascites tumor 1×10^6 cells/ml were incubated with Ce6 and CuChlNa at various concentrations and subjected at 15 min to the photodynamic irradiation from a semiconductor laser with 32 mW output power at the light wavelength of 650 nm, giving an irradiation dosage of 6 J/cm^2 . Intracellular uptake of CuChlNa and Ce6, phototoxicity, apoptosis and necrosis of cells after incubation at 9 days was studied. Were shown, that CuChlNa is able to more intense absorption at therapeutically relevant wavelengths. After 6-days exposure to CuChlNa more than $77 \pm 6.1\%$ of the cells apoptoses were detected, whereas in the same period after contact with Ce6 were indicated predominant cell necrosis. The therapeutic efficacy of photosensitizers has been shown in vivo by demonstrating the high survival rate of mice treated with CuChlNa and PDT.

Keywords: photosensitizer; chlorophyllin; Chlorin e6; cytotoxicity, intracellular localization; Ehrlich' s ascites tumor; photodynamic therapy; oncology

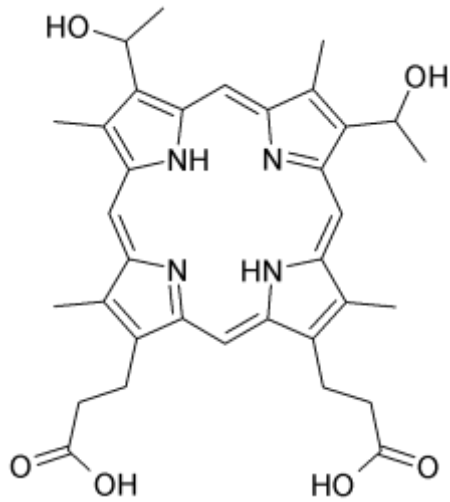


Figure 1. Hematoporphyrin structure

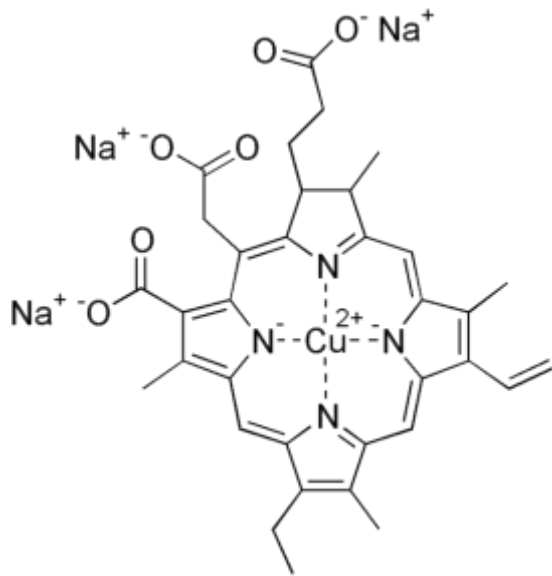


Figure 2. Chlorophyllin sodium-copper structure (CuChlNa)

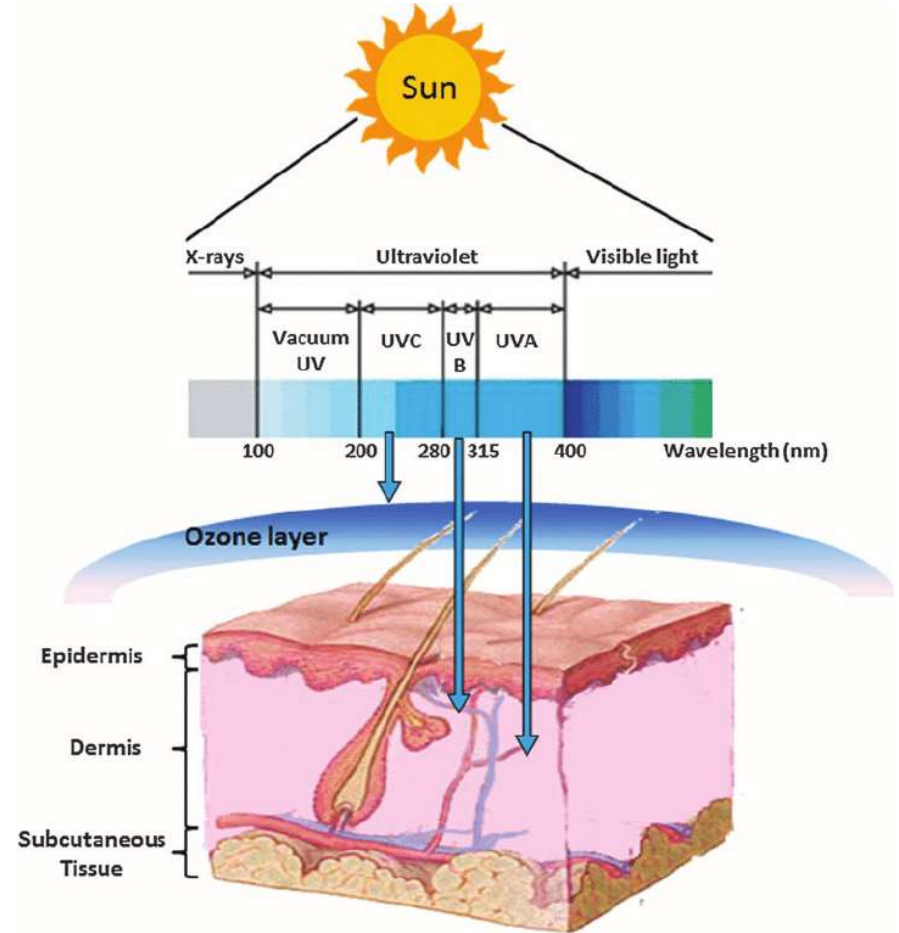
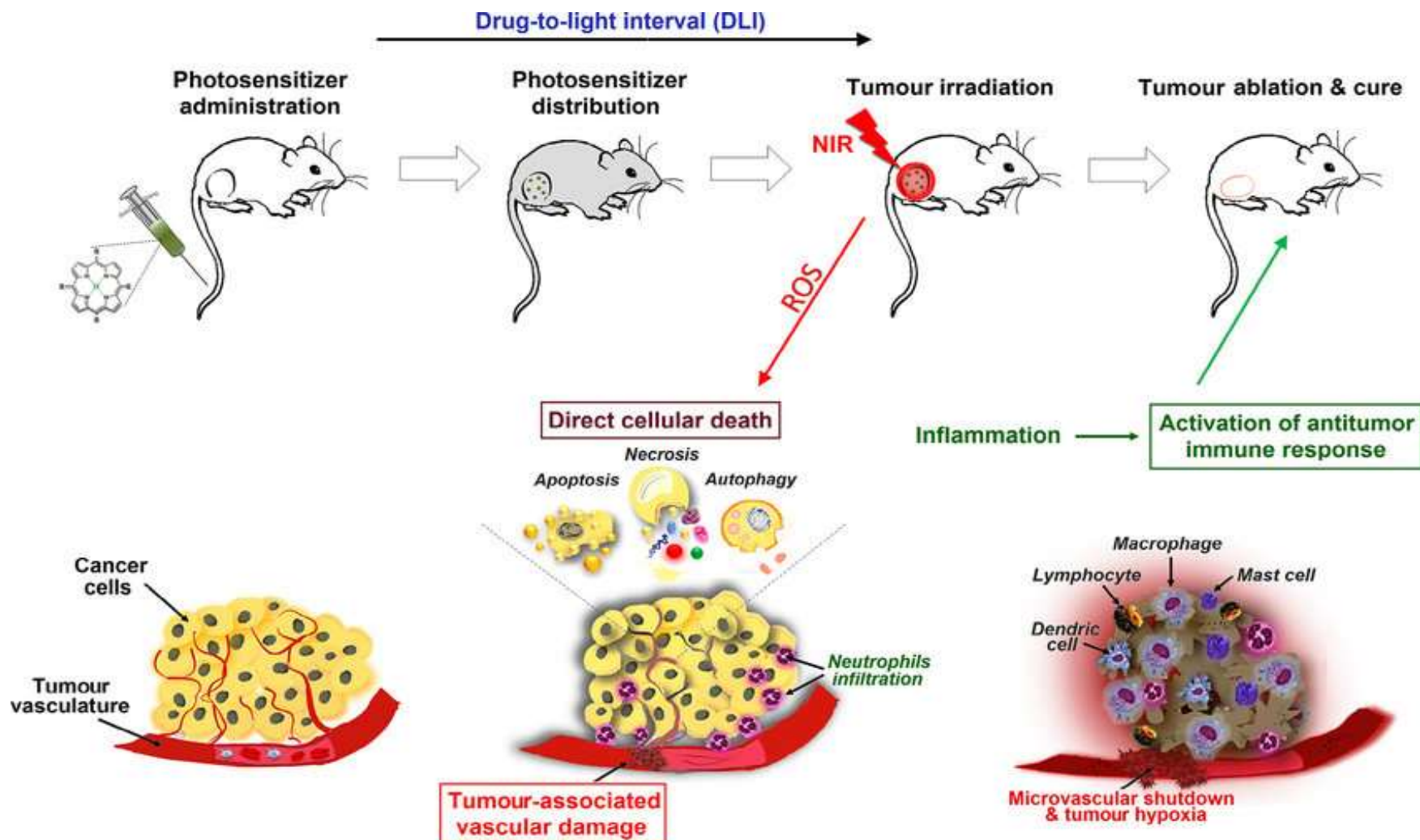


Figure 3. Depth of light penetration into tissue [1]

The principle of Photodynamic Therapy

Figure 4. Principles of photodynamic therapy: the steps of PDT procedure and biological mechanisms resulting in the direct and indirect elimination of tumours by apoptosis, necrosis, autophagy, vascular damage and inflammatory response.



Results and Discussion. Absorption spectra

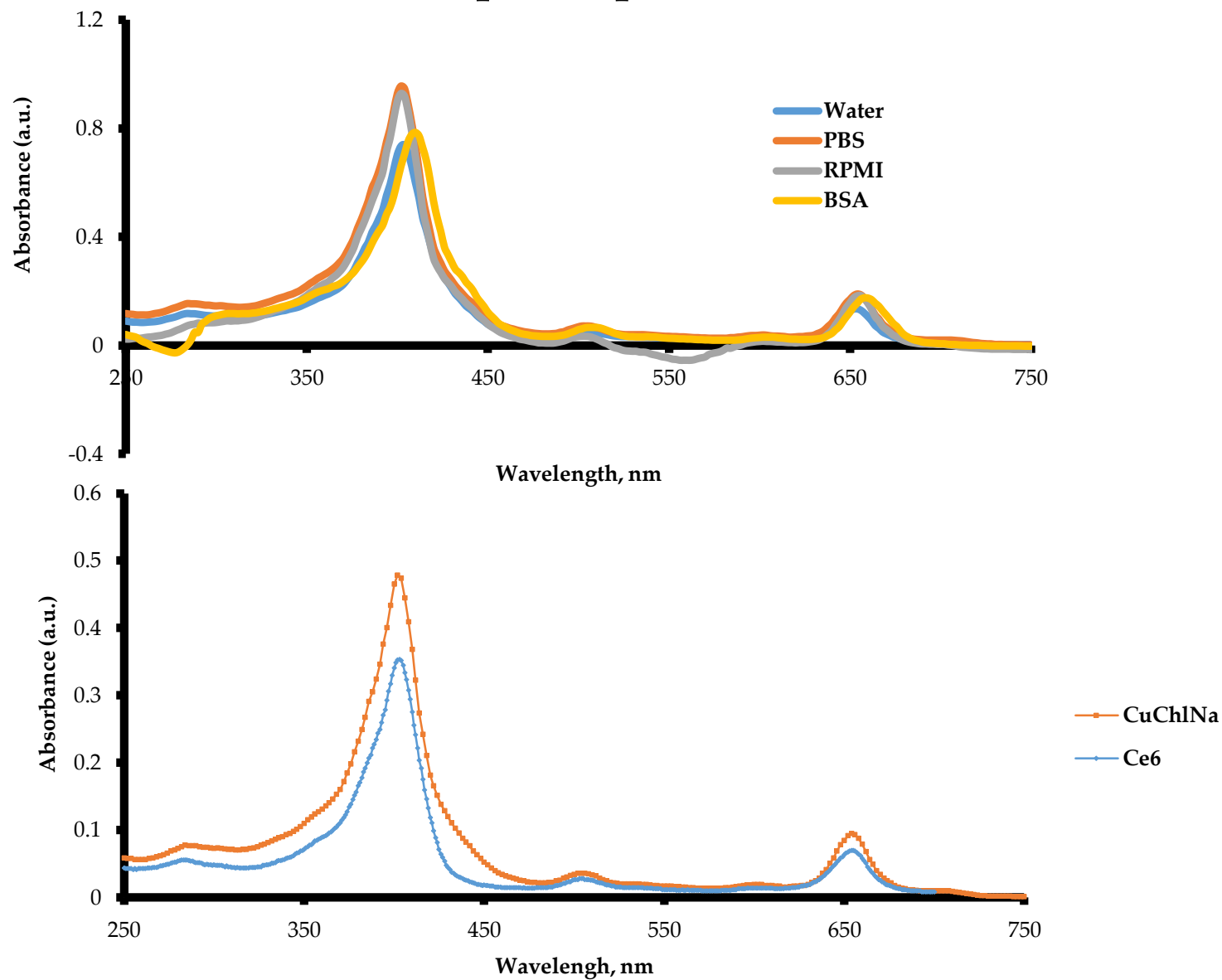


Figure 5. (A) The absorption spectra of Ce6 and CuChlNa (both 1 mg/m) (a) and (B) CuChlNa in various solvents .

Results and Discussion. *In vitro* phototoxicity assay

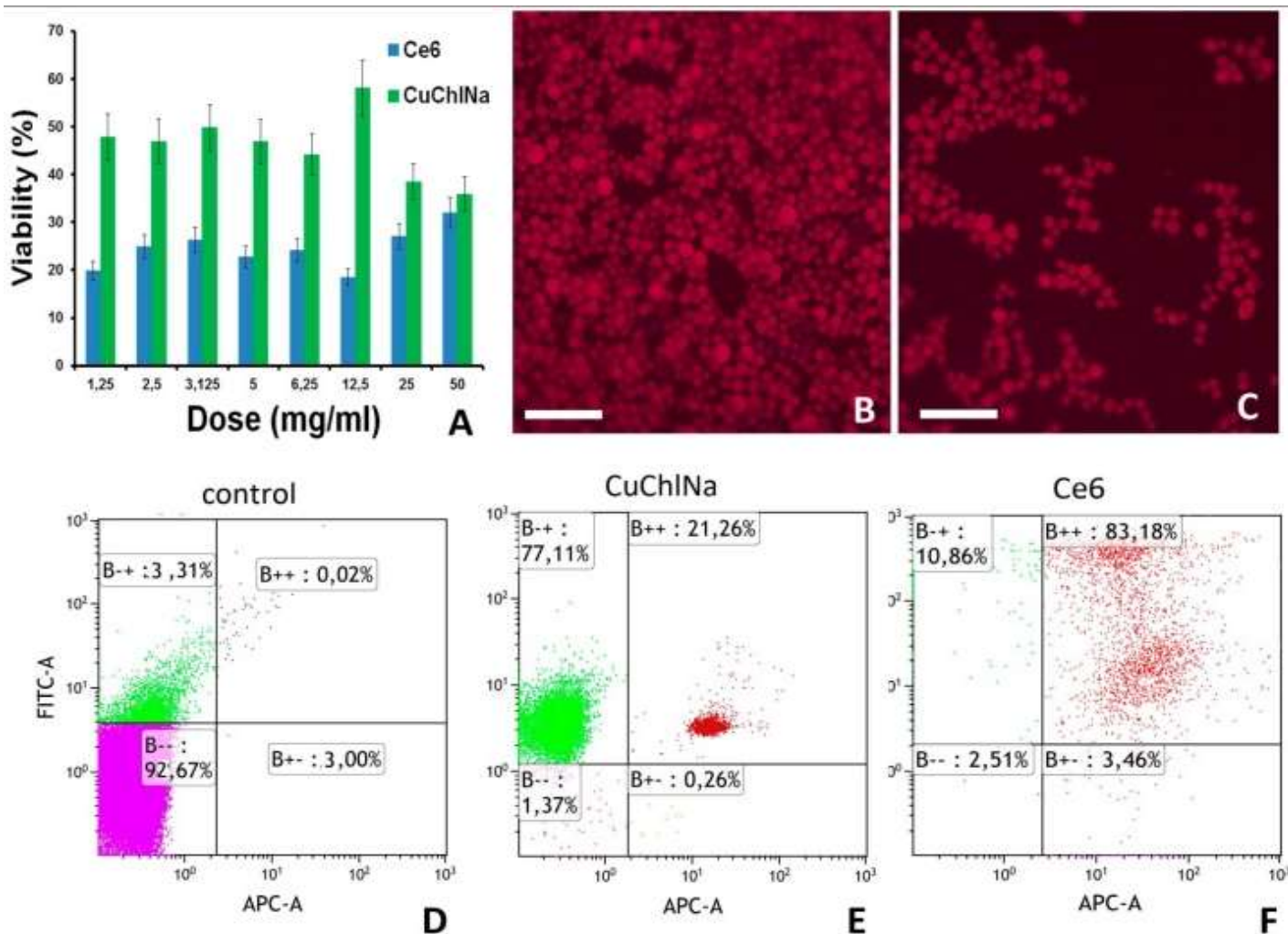


Figure 6. (A) EAT cells treated with Ce6 and CuChlNa (1.25 $\mu\text{g/ml}$) after incubated for 8 days. (B, C) The cell survival rates of EAT cells were assessed by the MTT assay; subcellular localization (red) of Ce6 and CuChlNa in EAT were detected with fluorescence microscope. (D-F) Scale bar, 25 μm . Cell apoptosis and necrosis of EAT cell was detected by the Annexin-V/PI staining and flow cytometry.

Results and Discussion.

Analyzing Cell Death by Nuclear Staining with Hoechst 33342

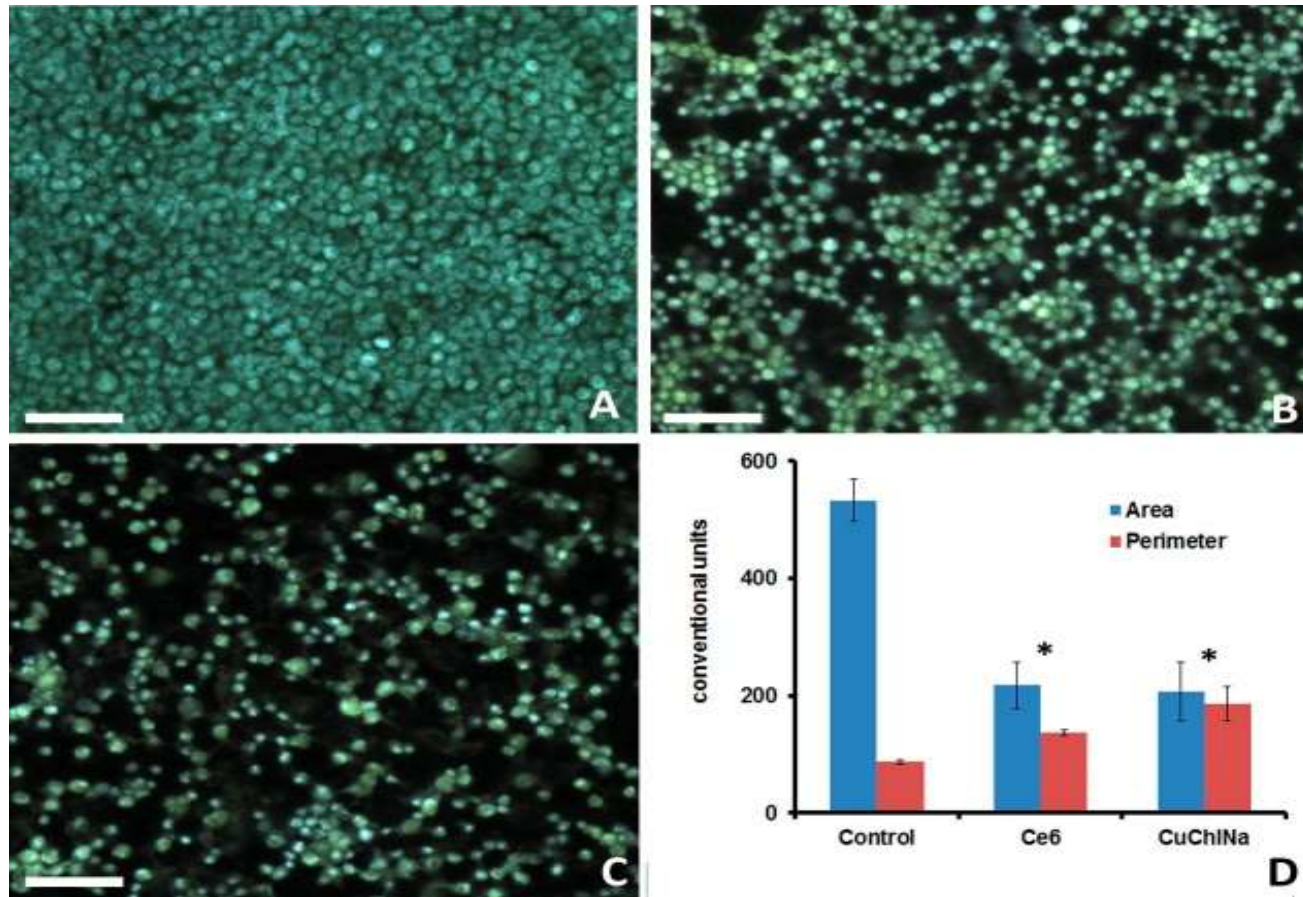


Figure 7. EAT cells treated with Ce6 and CuChlNa (1.25 $\mu\text{g/ml}$), after incubated for 6 days. (A, B, C) EAT cells stained Hoechst 33342 were detected with fluorescence microscope. Scale bar, 25 μm . (D) Statistical analysis of EAT cells sizes treated with Ce6 and CuChlNa and PDT. Compared with the corresponding control group without photodynamic irradiation, * $P < 0.05$

Results and Discussion. *In vivo* therapeutic efficacy of PSs

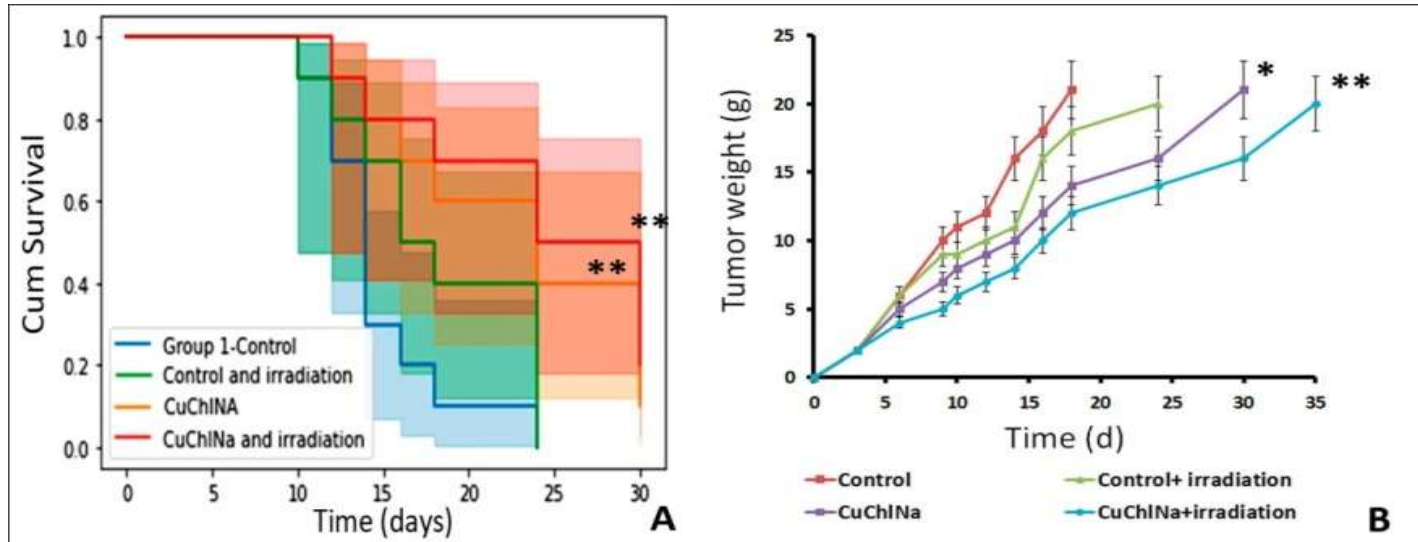


Figure 8. (A) Kaplan-Meier survival curve of mice treated with saline, saline plus Tween and CuChlNa (7.25 $\mu\text{g}/\text{ml}$) (N = 10 in each group) (a). (B) Effect of Ce6 and CuChlNa (7.25 $\mu\text{g}/\text{ml}$) on tumor weight in mice. * $P < 0.05$ and ** $P < 0.01$ compared with control group.

Conclusions

Natural PS isolated from plants can be seen as a green approach to PDT in cancer therapy. Low systemic cytotoxicity in relation to normal cells and selective action against malignant cells is one of the main advantages of natural PS for PDT. In addition, since natural compounds are commonly found ubiquitously, they may be more readily available compared to synthetic chemotherapeutic agents. Researchers are now able to discover these photoactive plants in nature and compare the effects with more traditional PSs. In addition, loading conventional PSs or natural phototoxic agents into nanostructures can help achieve better cancer treatment with PDT.

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The reported study was funded by PSMU «Evaluation of anti-tumor activity photosensitizers of the porphyrin class»



Thank You for attention!

