Journal of Oil Palm Research
DOI: https://doi.org/10.21894/jopr.2023.0006

ANTIPROLIFERATIVE EFFECTS OF PALM OIL IN THE PRESENCE OF PHOTOBIOMODULATION AGAINST K562 CANCER CELLS

AZADEH HEKMAT[1]*; KIMIA FAROKHI BAHAR[1] and ZAHRA HAJEBRAHIMI[2]

ABSTRACT

Palm oil (PO) is utilised for food such as cooking oil and non-food such as for making creams, soaps and detergents. It contains various unsaturated and saturated fats, vitamin E, and β-carotenes. This study aimed to clarify the influence of PO plus photobiomodulation (PBM) on leukaemia (K562 cells) proliferation. Cells were treated with various concentrations of PO, PBM at wavelength 655 nm with 1, 2, 3 and 6 J/cm^2, and PO in pre- and post-irradiation with PBM. The proliferation of cells was investigated by MTT assay, morphologic microscopy and flow cytometry. The amount of reactive oxygen species (ROS) was also determined. Cells were subjected to PO and then PBM in the presence of NAC (N-acetylcysteine) to assess the involvement of ROS in cell growth. PO can diminish the viability of K562 cells significantly. PBM did not have a remarkable effect on the viability of cells. Pre-treatment with PO and then irradiation with 1 J/cm^2 energy could induce apoptosis through intracellular ROS generation and had effective antiproliferative impacts on cells compared to those which acquired separate treatments with laser irradiated alone or PO alone. Thus, our research offers new strategies to utilise PO in combination with PBM in cancer treatment.

Keywords: flow cytometry analysis, K56 leukaemiaia cells, MTT assay, palm oil, photobiomodulation (PBM).

Received: 10 April 2022; **Accepted:** 31 October 2022; **Published online:** 24 February 2023.

INTRODUCTION

Organic compounds found in natural sources (microorganisms, animals, as well as plants) have been an inspiration for drug development. Various plant-derived compounds have been utilised as anticancer agents, for example, paclitaxel (PTX) was found in *Taxus brevifolia* and *Taxus baccata* (Pashah *et al*., 2019). *Elaeis*, a genus of palms, contains three accepted species. The first two, *E. oleifera* (also named *E. melanococca*), and *E. guineensis* are the American and African palms, respectively. The third species, *E. odora*, is not cultivated and there is little information about it (Godswill *et al*., 2016). *Elaeis* originated from the Greek word erlaion, meaning oil. Palm oil (PO) is obtained from the fleshy mesocarp of the fruit which contains 45%-55% oil, however, the colour varies from orange-red to light yellow. PO melts at 25°C and is utilised for cooking and making creams and soaps, and administered as a poison antidote (Owoyele and Owolabi, 2014; Parveez *et al*., 2021). It is also utilised with various other herbs as a lotion for skin diseases (Ahmad *et al*., 2021). It is well-known to be effective against various forms of intestinal disorders, particularly dysentery in infants and diarrhea (Owoyele and Owolabi, 2014). PO contains various unsaturated and saturated fats in the forms of palmitate (44.0%, saturated), oleate (39.0%, monounsaturated), linoleate (10.0%, polyunsaturated), stearate (5.0%, saturated), myristate (1.0%, saturated), alpha-linolenate (0.3%, polyunsaturated), and glyceryl laurate (0.1%, saturated) (Owoyele and Owolabi, 2014). It also offers a rich source of vitamin E and β-carotenes, such as tocotrienols and tocopherols which are recognised nutritional antioxidants that behave as scavengers of free radicals or the oxygen atom. Previous studies have confirmed the antioxidant, anti-diabetic, anti-bacterial, anti-cancer and anti-inflammatory effects

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[2] A&S Research Institute, Ministry of Science Research and Technology, Tehran, Iran.

* *Corresponding author e-mail: hekmat@ut.ac.ir*

of PO (Owoyele and Owolabi, 2014). It has been shown that PO phenolics enhanced the growth of BHK normal cells (Syrian Baby Hamster Kidney) (Sekaran *et al.*, 2010). However, more *in vitro* findings have revealed that the lipid-soluble compounds found in PO, particularly tocotrienols, can induce apoptosis and inhibit proliferation in different human cancer cells including lung (Ji *et al.*, 2012), melanoma (Komarasamy and Sekaran, 2012), pancreatic (Ji *et al.*, 2015), breast (Sekaran *et al.*, 2010) and prostate (Kumar *et al.*, 2006) cancers. Correspondingly, in animal models, it was discovered that PO phenolics can reduce tumour growth (Sambanthamurthi *et al.*, 2011; Sekaran *et al.*, 2010).

Photobiomodulation therapy (PBMT) also known as low-level laser therapy (LLLT) is a fast-growing technology utilised to modify cellular behaviours or tissue regeneration. The PBMT mechanism is hinged directly on the biomodulation effect or biostimulation, implying that irradiation at a certain wavelength could modify cellular behaviours (Kara and Orbak, 2009). PBMT can induce reactive oxygen species (ROS) in a cancer cell (Kara and Orbak, 2009). It has been known that wavelengths in the range of 390-600 nm can be utilised to treat superficial tissue, and longer wavelengths in the range of 600-1100 nm, which penetrate further, can apply to treat deeper-seated tissues (Avci *et al.*, 2013).

Chronic Myelogenous Leukaemia (CML) and several other types of cancer are recognised to develop resistance to existing drugs through numerous resistance mechanisms, such as drug efflux caused by poor drug permeability and P-glycoproteins, deregulation of mismatch repair mechanism, mutations, variations in the metabolic pathways as well as alternative drug export pumps (Robey *et al.*, 2018). Resistance to antitumor drugs and their harmful adverse effects on cancer patients, induced the scientific community to design novel cancer treatments. Consequently, we were motivated to clarify the influence of PO in the presence of PBM at a wavelength of 655 nm on chronic myelogenous leukaemia (K562 cells) proliferation by MTT assay, morphologic microscopy and flow cytometry. The amount of ROS was also determined. Cells were also subjected to PO and then PBM in the presence of NAC (*N*-acetylcysteine) to assess the involvement of ROS in K562 cell growth. All of the evidence acquired from this research could offer an advantageous and innovative clinical strategy for cancer therapy in the future.

MATERIALS AND METHODS

Materials

The K562 human chronic myeloid leukaemia cell line (ATCC No. CCL-243™) was acquired from the Iranian Biological Resource Center, Iran. MTT (3-[4,5-Dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide), DCFH-DA (2′,7′-Dichlorodihydrofluorescein diacetate), and DAPI (4′-6-Diamidino-2-phenylindole) were acquired from Sigma Aldrich Co., USA. PO produced by Mewah Oils & Fats Pte Ltd. Co., Malaysia, bought from Behshahr Industrial Co., Iran. The fetal bovine serum (FBS) and RPMI-1640 medium were prepared from Gibco, USA. Dimethylsulfoxide (DMSO) and NAC were obtained from Merck, Germany. Streptomycin and penicillin were obtained from Bio-idea, Iran. The Annexin FITC kit was obtained from IQ product, Netherlands.

Palm Oil Sample Preparation

The stock solution was prepared by dissolving 500 μg of palm oil in 1 mL ethanol (70% v/v) then the solution was shaken gently in a shaker for 15 min. For cell viability measurements, ROS level measurements, and NAC treatment, 1 μL from stock solution was taken and diluted to 99 μL culture medium and obtained the first dilution contained 5 μg/mL of palm oil. In the same way, dilutions containing 10, 25, 50, 75 and 100 μg/mL of palm oil were prepared. For each concentration of palm oil, 10 samples were prepared and tested. For cell morphological observation and flow cytometry method, 6 μL from stock solution was taken and diluted to 599 μL culture medium and obtained the first dilution containing 5 μg/mL of palm oil. In the same way, dilutions containing 10, 25, 50, 75 and 100 μg/mL of palm oil were prepared. All sample solutions were sterilised by filtration through a filter (0.22 μm pore size). All solutions obtained were transferred to amber bottles and kept until use. To investigate the biological effects of ethanol (used for dissolving palm oil), 1, 2, 5, 10, 15 and 20 μL of 70% v/v ethanol were diluted with a medium to prepare 100 μL of 0.7%, 1.4%, 3.5%, 7.0%, 10.5% and 14.0% v/v ethanol, respectively.

In vitro Laser Irradiation

In this study, for *in vitro* laser applications, a "red" diode laser (PFA 23847V, Pooya Far Azma Co., Iran) with a wavelength of 655 nm and a cooling system were utilised under the following irradiation parameters: Power 220 V, frequency 50/60 Hz, continuous wave mode, and 1 μm beam radius. Plates with 96 wells each were utilised, and the cells were seeded at an initial density of 5×10^4 cells/cm^2 in wells. Irradiation was cautiously measured and performed in a dark laminar flux hood. Furthermore, to prevent any overlapping of the irradiated light caused by scattering K562 cells were seeded separately and a black plate was utilised

as a background to diminish specular reflection. A schematic representation of the 96-well plates made for cell proliferation and PBM applications is demonstrated in *Figure 1*. After 24 hr, the cells were exposed to laser irradiation. The effective power was measured in cell culture plate wells utilising an advanced laser power meter (Pooya Far Azma Co.). The energy intensity applied to cells was adjusted to approximately 1, 2, 3 and 6 J/cm^2 by applying 30 mW power for 30, 60, 90 and 180 s, respectively. The irradiating probe was kept vertically over each well at a total distance of 1 cm above the cells.

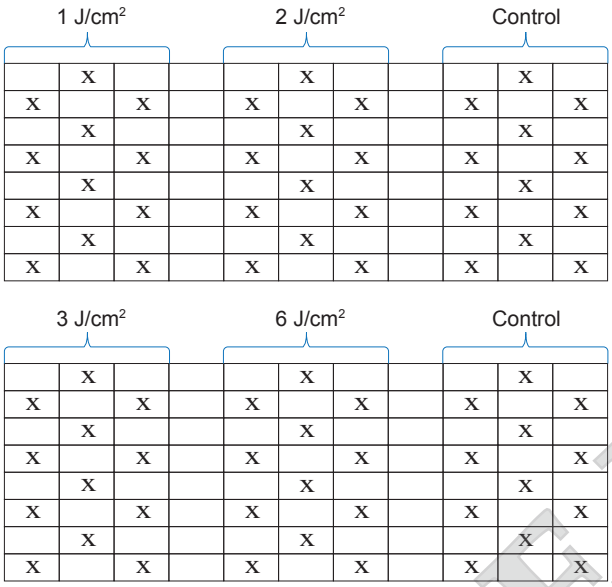

Figure 1. The schematic representation of the 96- well plates for PBM applications.

Cell Culture and Treatment

Cells were cultured in RPMI 1640 medium including FBS (10%, heat-inactivated) and 1% Pen Strep (10 000 units/mL Penicillin and 10 mg/mL Streptomycin) in a humidified incubator including 5% CO_2 at 37°C. Upon reaching more than 90% confluence, cells were subcultured. The K562 cells were divided into five groups randomly, including one control group and four experimental groups. The experimental groups were (1) cells irradiated with 1, 2, 3 and 6 J/cm^2 for 30, 60, 90 and 180 s, respectively, (2) cells treated with the sterilised PO in various concentrations (5, 10, 25, 50, 75 and 100 µg/mL) for 24 hr, (3) cells were irradiated with 655 nm (1 J/cm^2; for 30 s) after that treatment with the PO (5 µg/mL) for 24 hr, (4) cells were treated with the PO (5 µg/mL) for 24 hr then exposed to PBM (1 J/cm^2; for 30 s).

Measurement of Cell Viability

After 24 hr treatment of K562 cells (control and experimental groups), 0.5 mg/mL MTT (20 µL) was added to each well of 96-well plates. Subsequently, the cells were incubated for 3 hr in a CO_2 incubator at 37°C. Later, the insoluble formazan formed was dissolved in 100 µL of DMSO and mixed (Dashtaki *et al.*, 2020; Ibrahim *et al.*, 2021). The OD (optical density) of each well at 570 nm, was measured against a reagent blank with an ELISA reader, ELx808, BioTek Instruments, Inc., USA. All trials were repeated 4 times.

Cell Morphological Changes/Fluorescence Imaging

Observation of morphological variations of K562 cells was done according to the previous method (Rahman *et al.*, 2013). After 24 hr of treatment of K562 cells with PO and/or PBM, the medium of each cultured cell was removed and washed once with 2 mL of cold PBS buffer (0.01 M, pH 7.4). Consequently, the cells were imaged utilising an inverted microscope, INV100; BEL Engineering, Italy. For DAPI staining, 4×10^5 cells/well were seeded in 24-well plates and treated with PO and/or PBM. Then, the cells were washed with cold PBS. DAPI solution was added to the cell suspension at a final concentration of 100 µg/mL. The cellular morphology was evaluated via fluorescence microscopy, Axoscope 2 plus, ZEISS, Germany.

Determination of Intracellular ROS Production

An oxidation-sensitive fluorescent probe, DCFH-DA, was employed to determine the formation of ROS based on the ROS-dependent oxidation of DCFH-DA to DCF (2′7′-dichlorofluorescein). Consequently, K562 cells were exposed to PBM (1 J/cm^2 for 30 S) (first experimental group); then cells were treated with the sterilised PO (5 µg/mL) for 24 hr (second experimental group). The other group was pre-treated with the sterilised PO (5 µg/mL) for 24 hr and then exposed to PBM. After that, the cell culture medium was picked up and the cells were incubated with 2 µM DCFH-DA for 45 min at 37°C. Later, the cells were washed with PBS and the OD of each well at 530 nm, was measured against a reagent blank with an ELISA reader, Elx808, BioTek Instruments, Inc., USA. All trials were repeated 3 times.

Antioxidant (NAC) Treatment

The impact of PO in the presence of the PBM on ROS generation in the absence or presence of NAC was investigated. NAC powder was solved in RPMI 1640, and then K562 cells were pre-treated with 4 mM NAC for 3 hr in a CO_2 incubator at 37°C. Later the cells were pre-treated with the sterilised PO (5 µg/mL) for 24 hr and then exposed to PBM. After 24 hr of treatment of cells, 0.5 mg/mL MTT

(20 µL) was added to each well of 96-well plates. The cell viability was examined according to the MTT assay as mentioned earlier.

Flow Cytometry Method

The Annexin V binding was utilised via the Annexin FITC kit. K562 cells were seeded into 6-well plates (10^6 cells/well) for 24 hr, then, treated with PO and/or PBM. Afterwards, the cells were collected through centrifugation for 5 min at 1000 g and washed twice with PBS buffer (0.01 M, pH 7.4). Then, the cells were suspended in 100 µL of Annexin V binding buffer (HEPES/NaOH (10 mM, pH 7.4), NaCl (140 mM), and 2.5 mM $CaCl_2$). Afterward, the cells were double-stained with a 5 µL solution of PI (Propidium iodide) and a 5 µL solution of FITC-labelled Annexin V. All trials were incubated for 30 min in the darkness at room temperature and later analysed via flow cytometry, BD FACSCalibur™, BD Biosciences Inc., USA.

Statistical Analysis

FlowJo software (Version 7.6.1.) was utilised for the analysis of flow cytometry data. Significant differences were evaluated by a t-test of GraphPad Prism Software (Version 8.4.3, GraphPad Software Inc., San Diego, USA). GraphPad Prism also was utilised for plotting graphs. All data were stated as the mean ± the standard deviation (SD). The $p<0.05$ (signified by a single asterisk (*)) was cidered statistically significant while $p<0.01$ (symbolized by a double asterisk (**)) and $p<0.001$ (symbolized by a triple asterisk (***)) were considered as statistically highly significant.

RESULTS AND DISCUSSION

Growth Rates of K562 Cells in the Presence of PO or/and PBM

As mentioned earlier, PO is rich in phytonutrients, and in folklore medicine, it is utilised in the treatment and management of cancer (Loganathan *et al*., 2017). In this study, since cell cultures are one of the best biological systems utilised to discover the impact of toxic compounds or drugs on the rate of cell proliferation, at first, the MTT assay was utilised to measure the influence of PO in the presence of pre- and post-exposed to PBM on K562 cells growth. At first, the effects of PO in various concentrations were examined on the growth of K562 cells under dark conditions. As demonstrated in *Figure 2a*, the relative viability of K562 cells decreased significantly. Thus, our data demonstrate that PO (particularly at higher concentrations) has the potential ability to restrain the proliferation of chronic myeloid leukaemia. This observation is consistent with previous *in vitro* and *in vivo* studies, which demonstrate that PO and its derived product have a significant role against cancer cells (Absalome *et al*., 2020; Loganathan *et al*., 2021; Sambanthamurthi *et al*., 2011). The underlying process of cell death caused by PO has been evaluated by some researchers. For example, Ji *et al*. (2015) reported that PO phenolics could generate apoptosis in pancreatic cancer cell lines (BxPC-3 and PANC-1) by suppression of the NF-κB pathway, *i.e*., with increased expression of cleaved PARP, and caspase-9, and caspase-3 with a reduction in expressions of Bcl-xL and survivin. PO contains vitamin E which is a fat-soluble antioxidant and contains two major categories, the tocotrienols, and the tocopherols (Abdullah *et al*., 2021). The anticarcinogenic ability of α-, β-, γ- and δ-tocotrienols has been shown in various cancers. These antitumor properties of tocotrienols were once presumed to owe to their antioxidant properties, however, recent data indicated that tocotrienols can influence numerous signalling pathways (Abdullah *et al*., 2021). Thus, although the mode of action of PO on cancer cells is not fully clear, however, possible routes of action for PO are via modulating antioxidants balance and oxidative stress within the cancer cells as well as influencing signalling pathways.

Even though nearly all solvents are toxic to cancer cells *in vitro*, they are still required for dissolving drug agents for biological assays. Hence, it is required to verify the most suitable concentrations of solvents to utilise in biological assays. Accordingly, since the stock solution was prepared by dissolving PO in ethanol, the effects of ethanol on K562 cells were investigated at the concentration of 0.7%, 1.4%, 3.5%, 7.0%, 10.5% and 14.0% (v/v). As shown in *Figure 2b*, ethanol showed non-toxic effects at concentrations of 0.7%, 1.4%, 3.5% and 7.0% (v/v). However, ethanol diminished K562 cell proliferation at higher concentrations (10.5%, and 14.0% (v/v)) considerably. Thus, the antiproliferative properties of PO at higher concentrations are influenced by concentrations of ethanol. Hence, in the follow-up research, PO at 5 µg/mL concentration (including 0.7% (v/v) ethanol) was selected.

PBM can define the variations in cellular activity in response to irradiation with light under particular conditions. PBM has offered an exciting new frontier in oncology (Tam *et al*., 2020). From the previous studies, it is apparent that exposure to various doses and wavelengths of laser can generate stimulation, inhibition, or even no effects at all in both cancerous and normal cells (Tam *et al*., 2020). Thus, in the next experiments, the impact of various laser irradiation energies at 655 nm on cells was investigated (*Figure 2c*). The results of the MTT assay showed that only at higher radiation energy (6 J/cm^2) and longer time of irradiation (180 s), cell survival was reduced slightly, *i.e*., lower energy level (1 J/cm^2)

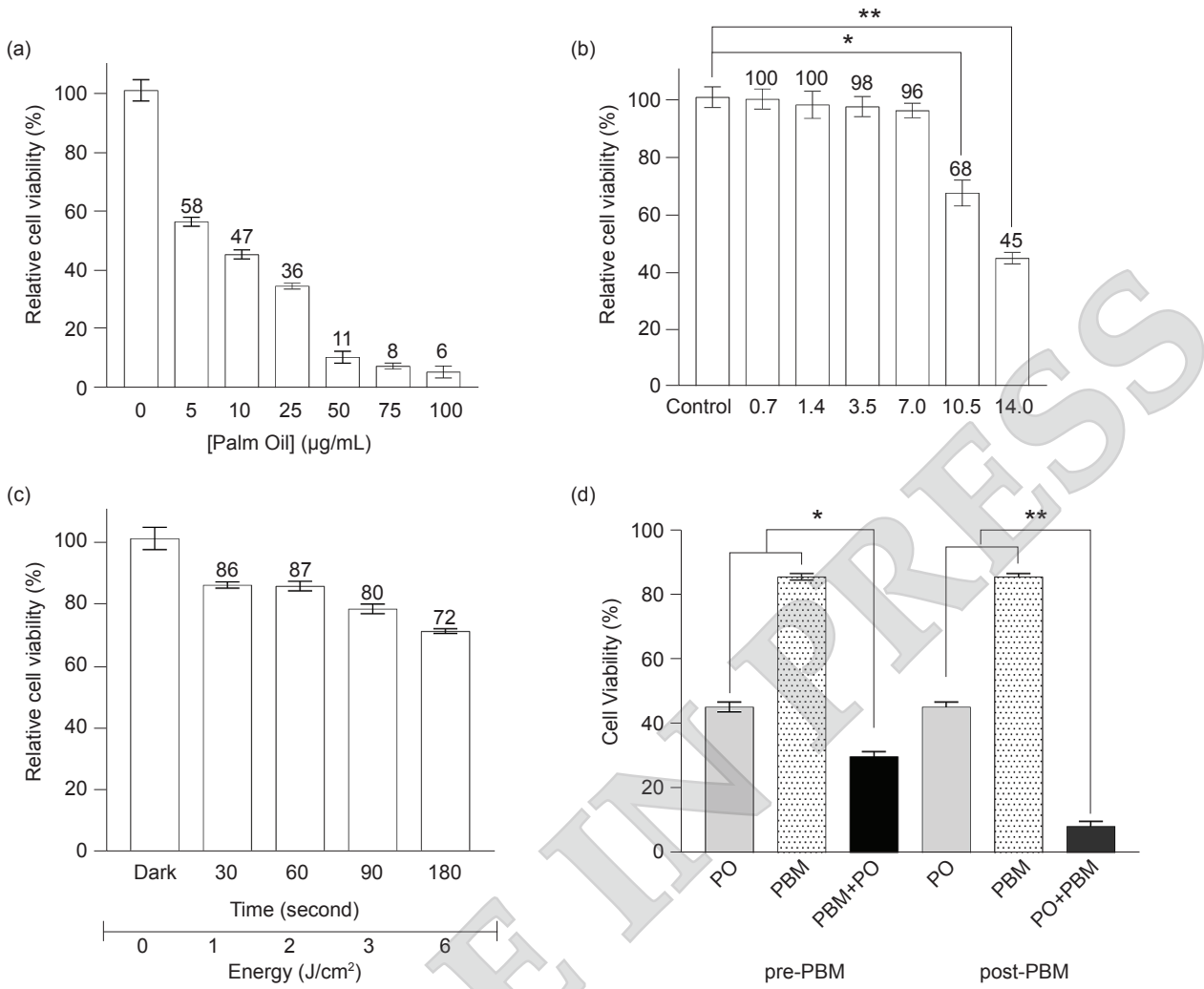

*Figure 2. MTT assay of K562 cells after treatment with (a) various concentrations of PO, (b) ethanol at the various concentrations (v/v%), (c) PBM in different duration of time and (d) PO in pre- and post-exposed to PBM. Values are mean ± standard deviation; * and ** indicate* $p<0.05$ *and* $p<0.01$*, respectively.*

did not have a remarkable effect on the viability of K562 cells. Numerous laboratory experiments have reported that dosage, tissue types and laser properties are important parameters in PBM. It has been also observed that the probability of getting a response in cells obtaining higher doses is larger compared to those obtaining lower doses (Tam *et al.*, 2020). Accordingly, this study displayed that PBM (655 nm, energy densities from 1 to 6 J/cm^2) alone is not capable of killing human chronic myeloid leukaemia cells (K562 cell line) significantly. Our observation is similar to the results of AlGhamdi *et al*. (2012) who published that lower doses of PBM (632.8 nm, energy densities from 0.5 to 4.0 J/cm^2, and power densities from 1-500 mW) did not have negative effects on the cell proliferation rate and other cellular functions. Our result is also consistent with the findings of Dastanpour *et al*. (2015) who found that lower energy densities (5 and 10 J/cm^2) did not exhibit any considerable variations in KG-1a cell proliferation. Even though several studies had been conducted, the full impact of PBM on cell life processes has not been identified completely. However, it has been shown that PBM can increase ATP production in cell cultures and also can induce mitochondrial retrograde signalling (Rola *et al*., 2022). Furthermore, based on the current state of knowledge, only higher energy density can promote apoptotic processes (Rola *et al*., 2022).

In the follow-up research, the cytotoxicity impact of PO in the presence of pre- and post-exposed to PBM was assessed (*Figure 2d*). Accordingly, PO at a concentration of 5 µg/mL and PBM with an energy level of 1 J/cm^2 were selected for the remaining experiments. It should be noted that, even though PBM at the energy level of 1 and 2 J/cm^2 generated the same variation in the cell viability, 1 J/cm^2 was chosen for the combined treatment owing to its minimum power to avoid any undesirable heat generation. Also, as miniated earlier the antiproliferative properties of PO at this concentration were not influenced by concentrations

of ethanol. When K562 cells were pre-exposed to PBM and then treated with PO for 24 hr, 30% cell growth was observed ($p<0.05$). However, when cells were treated with PO for 24 hr and then post-exposed to PBM only 5% cell growth was observed ($p<0.01$). Hence, our study demonstrated that although PBM alone is not capable of killing K562 cells significantly, however, the application of PBM can somehow enhance the cellular penetration of K562 cells into PO. Therefore, the anticancer effect of PO improves, significantly. It could be proposed that in the presence of red-light irradiation, phototoxic reactions sensitized tumour cells to PO and therefore, diminished the cell viability. Our results are similar to the results of Lee *et al.* (2020) who explored the influence of PBM (25 mW and 30 s irradiation time) combined with phloroglucinol on fibrosis inhibition *in vitro*.

K562 Cells Morphological Analysis

The K562 cells were evaluated for any altered morphology after being subjected to PO, PBM, and PO in combination with PBM utilising a light microscope. As seen in *Figure 3a*, control cells were round. Furthermore, the morphology of K562 cells treated with PBM with radiation energy of 1 J/cm^2 did not change (*Figure 3b*). However, the morphologies of cells treated with PO (under dark conditions) changed remarkably (*Figure 3c*). After being pre-treated with PO and then exposed to PBM, the number of cells remarkably reduced, cells were aggregated, and apoptotic bodies and parts of the condensed cytoplasm and nucleus were observable (*Figure 3d*). These results revealed the occurrence of apoptosis in the treated cells. According to these observations, it is clear that pre-treatment with PO and then irradiation with LLL can promote mortality of cells besides those mortality effects generated via PO alone.

To acquire a better insight into the influences of pre-treatment with PO and then irradiation, cell morphology was evaluated through the treatment of K562 cells with DAPI stain. The binding of DAPI with Thymine-Adenine bases in the minor groove of DNA can generate almost 20-fold fluorescence enhancement. As displayed in *Figure 4*, the viable cells are uniformly blue, while cells that were pre-treated with PO and then irradiated with 1 J/cm^2 are blue and contain bright blue dots in their nuclei, because of the nuclear fragmentation. This observation showed that pre-treatment with PO and then irradiation with LLL can induce nuclear fragmentation in K562 cells, which is a sign of apoptosis. This observation is in good agreement with MTT assay investigations as mentioned above.

Estimated ROS in K562 Cells After Treatments

Here we hypothesised that pre-treated with the sterilised PO and then exposed to PBM can alter ROS levels in cells. In this direction, the intracellular ROS level was analysed by the DCFH-DA probe. In *Figure 5*, K562 cells exhibited ROS production. The level of ROS increased significantly during treatment with the PO ($p<0.01$). However, the level of ROS produced using a 655 nm laser was not much higher than the non-irradiated cells. Nevertheless, the level of ROS significantly increased during pre-treated with PO and then exposed to PBM ($p<0.001$). It has

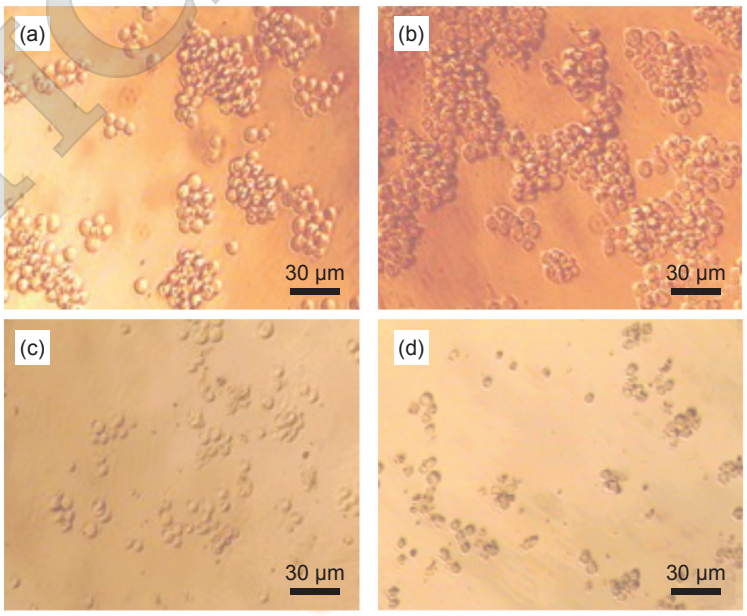

Figure 3. Morphological analysis of K562 cells: (a) control cells, (b) cells exposed to PBM (1 J/cm^2), (c) cells treated with PO (5 µg/mL) and (d) cells treated with PO in post-exposed to PBM (magnification 40x).

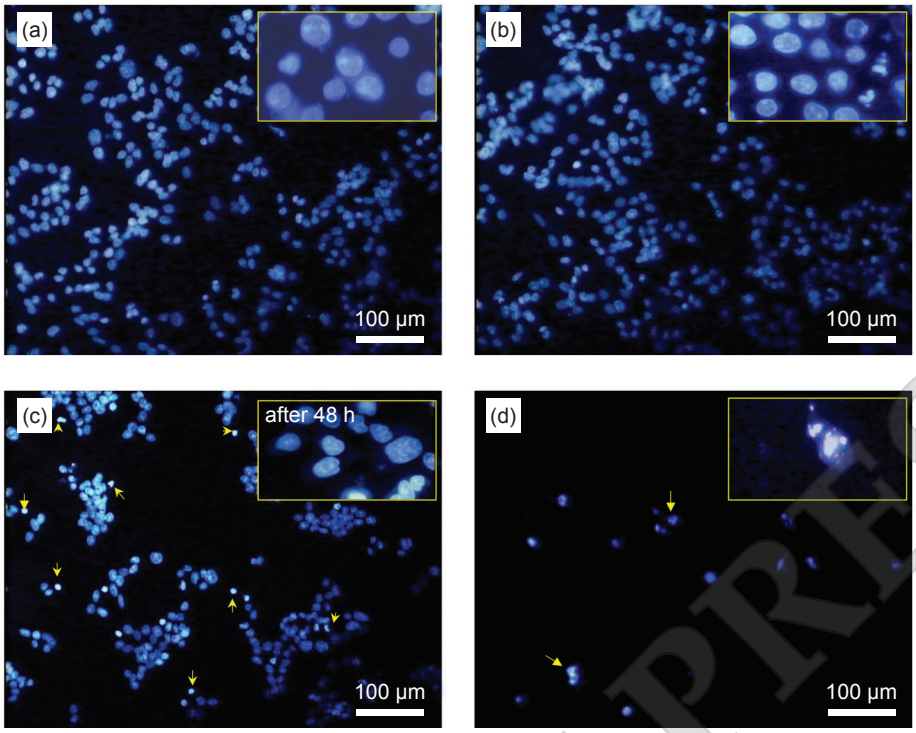

Figure 4. Fluorescence microscopic examination of K562 cells: (a) control cells, (b) cells exposed to PBM (1 J/cm²), (c) cells treated with PO (5 µg/mL) and (d) cells treated with PO in post-exposed to PBM. The cells were stained with DAPI. The yellow arrows point to destroyed chromatin in apoptotic cells.

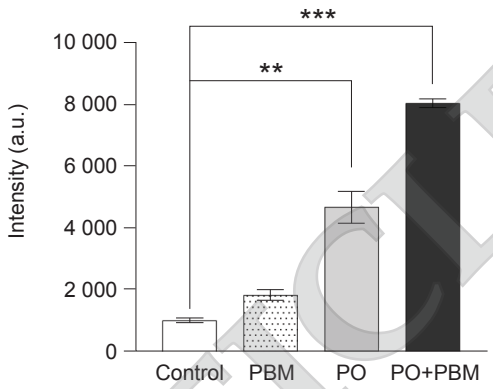

*Figure 5. ROS production level. K562 cells were pretreated with PO, PBM, and pre-treated with PO and then exposed to PBM. Values are mean ± standard deviation; ** and *** indicate* $p<0.01$ *and* $p<0.001$.

been shown that ROS can promote both extrinsic and intrinsic pathways of apoptosis in malignant cells. Most chemotherapeutic agents (such as doxorubicin, cisplatin, daunorubicin, topotecan and oxaliplatin) can produce ROS and altered redox homeostasis in malignant cells (Nakamura and Takada, 2021). Furthermore, several natural anticancer compounds (such as naringenin, epicatechin and catechin) can inhibit the protein kinase C signalling pathway and increase ROS generation which consequently causes ROS-mediated apoptosis (Bekhet *et al.*, 2021). Collectively, our results indicate that ROS is one of the effectors in the death mechanism of K562 cells by PO in the presence of PBM. At first sight, this observation seems to be in contrast to the proposed antioxidant role of PO (Arai *et al.*, 2022; Owoyele and Owolabi, 2014). However, as indicated by several studies, antioxidant compounds (such as resveratrol, campesterol, and quercetin) can display prooxidant activity in cancer cells at higher concentrations (Bekhet *et al.*, 2021). Hence, although experiments have reported on the ROS scavenging capacity of PO, however, we observed that PO could increase ROS production in K562 cells, and an increase in ROS production intensified in the presence of PMB.

Effect of NAC on Proliferation of PO+LLLI-Treated Cells

To discover whether oxidative stress could play a part in the cytotoxicity of PO and PBM, K562 cells were subjected to PO for 24 hr and then subjected to PBM in the presence of NAC. As indicated in *Figure 6*, compared with the control, pre-treatment with NAC eliminated the cytotoxicity of pre-treated with PO and then irradiated with LLL in K562 cells, noticeably ($p<0.001$). Although NAC (as an antioxidant) cannot scavage O_2 (superoxide radicals), however, can scavenge directly OH (hydroxyl radicals), HOCl (hypochlorous acid), and H_2O_2 (hydrogen peroxide). Furthermore, consistent with the literature, NAC can only scavenge intracellular ROS (Yan *et al.*, 2017). Therefore, even though the exact mechanisms are still unclear, our results imply that ROS is one of the effectors in

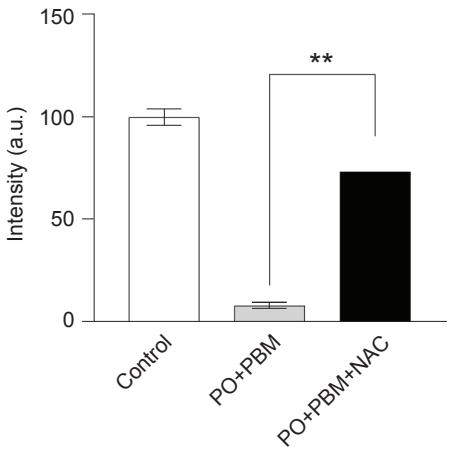

*Figure 6. Effect of NAC on PO+PBM-treated cells. K562 cells were pretreatment with NAC then 475 pre-treated with PO and later exposed to PBM. Values are mean ± standard deviation; **476 indicates* $p<0.01$.

the death mechanism of K562 cells by PO in the presence of PBM, *i.e.*, K562 cells undergo ROS-dependent cell death in response to irradiation and treatment with PO. ROS can generate the activation of various biological pathways, causing apoptosis, differentiation or cell proliferation. Further increment in intracellular ROS can lead to DNA structure modification, protein function alternation and changes in mitochondrial function (Perillo *et al.*, 2020). This observation is in good agreement with the result of ROS level estimation as mentioned above.

Influence of PO in the Presence of PBM on Necrosis or Apoptosis of K562 Cells

Apoptosis or programmed cell death is a significant mechanism to protect tissues and organs from several kinds of cell destruction and stress. Unlike necrosis, apoptosis does not initiate an inflammatory response, thus apoptosis induction in cancer therapy is very valuable (Hattori *et al.*, 2015). Flow cytometry is an appropriate instrument for the simultaneous assessment of apoptosis and necrosis in cells. Accordingly, the K562 cells were treated with PO, and PBM, as well as pre-treated with PO and then irradiated with PBM. Subsequently, the early-stage apoptosis, necrosis and late-stage apoptosis rates of cells were identified utilising the flow cytometry and Annexin V/PI double staining method (*Figure 7*). PI stains cells can only be identified in necrosis or late apoptosis, while Annexin V can be identified in both the late and early stages of apoptosis. Accordingly, living cells (Annexin V^-/PI^-, bottom left quadrant), early apoptotic cells (Annexin V^+/PI^-, bottom right quadrant), late apoptotic cells (Annexin V^+/PI^+, top right quadrant) and necrotic cells (Annexin V^-/PI^+, top left quadrant) were classified (Marasini and Aryal, 2022). As observed in *Figure 7b*, when cells were treated with PO, an increase in the content of both early and late apoptotic cells was observed (30.8% and 18.8%, respectively). However, the proportions of necrotic cells were 5.1%. On the contrary, no cytotoxic effect of PBM (1 J/cm^2) was observed in cells in comparison to the dark group, which agrees with the morphological and MTT cytotoxic results (*Figure 7c*). In contrast, after pre-treated cells with PO and then irradiated with LLL, a considerable increase in the content of both early and late apoptotic cells (56.7% and 25.5%, respectively) was detected (*Figure 7d*). It should be noted that compared with control cells no obvious necrotic cell death was identified in the culture. These data correlate with those of Razzaghi *et al.* (2021) who observed that the number of pre-apoptotic cells in A375 cells treated with liposomal doxorubicin (DOX) and then exposed to laser irradiation (λ=655 nm, 5 J/cm^2) was higher than that of cells treated with only liposomal DOX. More interestingly, our result correlates with those of Seragel-Deen *et al.* (2020) who have proved that higher apoptotic cells were observed in HEp-2 cells after pre-treatment with cisplatin and then irradiated with 190.91 J/cm^2 laser irradiation compared to those which acquired separate treatments with laser irradiated alone or cisplatin alone.

Taken together, based on our results, the cell death mechanisms of PO could be potentiated by utilising PBM possibly through ROS production and apoptosis induction. Pre-treatment of K562 cells with PO is thought to represent the initial signal to apoptosis, after that PBM floods the cell with ROS that further sensitizes the K562 cells to react to PO apoptotic stimuli. According to the literature, higher ATP levels would be consumed in apoptosis processes (Diniz *et al.*, 2020; Lee *et al.*, 2020). Hence the extra energy derived from PBM can increased the level of apoptosis in K562 cells. Therefore, PO in the presence of PBM could introduce a novel treatment for leukaemia. It is important to mention that even though cell culture is an essential method for understanding the probable cell reactions to treatment, however, one should be careful before generalising *in vitro* results, because cell behaviour and cell-matrix interactions in the complex environments of tissues can generate unexpected reactions. Thus, although the present *in vitro* study offers encouraging data, the exact mechanism remains undiscovered and ought to be clarified.

CONCLUSION

Briefly, we proved that pre-treatment with PO and then irradiated with 1 J/cm^2 energy could induce apoptosis through intracellular ROS generation and had effective antiproliferative impacts on the K562 cells compared to those which acquired separate treatments with laser irradiated alone or PO alone.

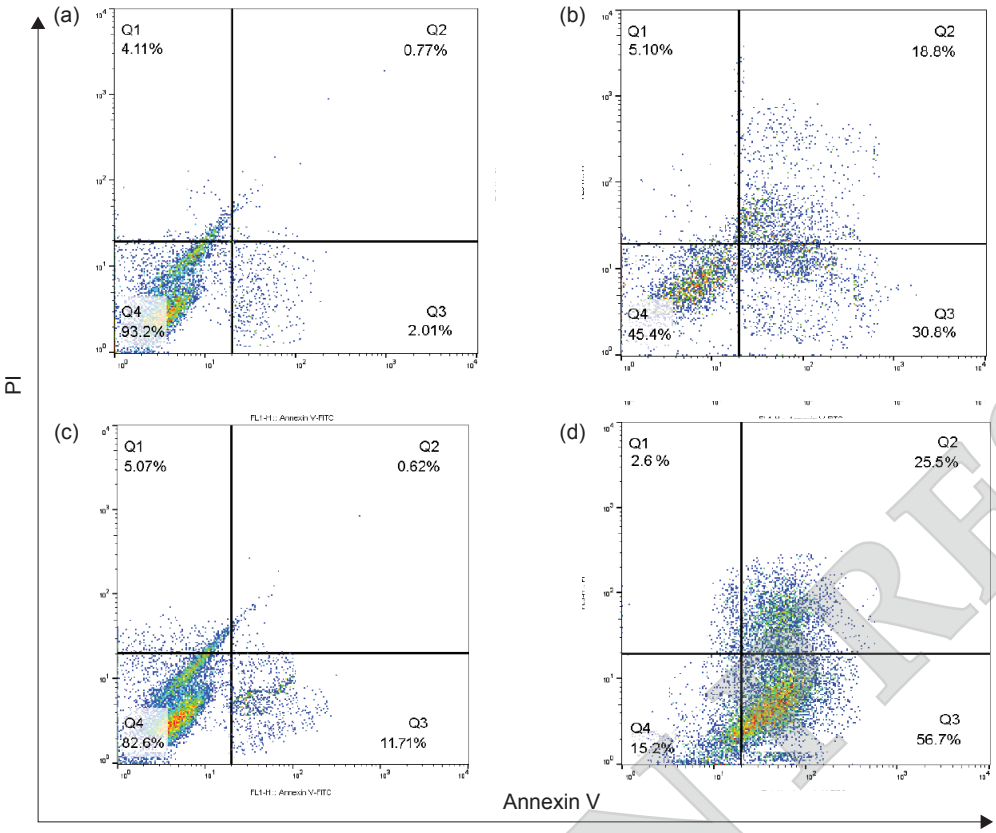

Figure 7. Flow cytometry analyses of K562 cells: (a) control cells, (b) cells treated with 5 µg/mL PO, (c) cells exposed to PBM (1 J/cm²) and (d) cells pre-treated with PO and then exposed to PBM.

Thus, even though further experiments are vital, our research offers new strategies to utilise PO in combination with PBM in cancer treatment.

ACKNOWLEDGEMENT

The authors appreciate and acknowledge all the people who helped us do this project.

REFERENCES

Abdullah, A; Atia, A; Alrawaiq, N S; Yusof, M K M and Rusli, M F (2021). Palm oil tocotrienols in cancer chemoprevention and treatment. *Elaeis guineensis* (Kamyab, H ed.) IntechOpen, London. p. 1-28. DOI: 10.5772/intechopen.98199.

Absalome, M A; Massara, C C; Alexandre, A A; Gervais, K; Chantal, G G A; Ferdinand, D and Brigitte, T (2020). Biochemical properties, nutritional values, health benefits and sustainability of palm oil. *Biochimie, 178*: 81-95. DOI: 10.1016/j.biochi.2020.09.019.

Ahmad, N; Hasan, Z A A and Bilal, S H (2021). Stability and performance of palm-based transparent soap with oil palm leaves extract. *J. Oil Palm Res., 33(4)*: 724-731. DOI: 10.21894/jopr.2021.0024.

AlGhamdi, K M; Kumar, A and Moussa, N A (2012). Low-level laser therapy: A useful technique for enhancing the proliferation of various cultured cells. *Lasers Med. Sci., 27(1)*: 237-249. DOI: 10.1007/s10103-011-0885-2.

Arai, W; Kameya, H; Hashim, R; Sulaiman, O; Arai, T; Sudesh, K and Kosugi, A (2022). Reactive oxygen species scavenging capacities of oil palm trunk sap were evaluated using the electron spin resonance spin trapping method. *Ind. Crops Prod., 182*: 1-9. DOI: 10.1016/j.indcrop.2022.114887.

Avci, P; Gupta, A; Sadasivam, M; Vecchio, D; Pam, Z; Pam, N and Hamblin, M R (2013). Low-level laser (light) therapy (LLLT) in skin: Stimulating, healing, restoring. *Semin. Cutan. Med. Surg., 32(1)*: 41-52.

Bekhet, O H and Eid, M E (2021). The interplay between reactive oxygen species and antioxidants in cancer progression and therapy: A narrative review. *Transl. Lung Cancer Res., 10(9)*: 4196-4206. DOI: 10.21037/tcr-21-629.

Dashtaki, A; Mahjoub, S; Zabihi, E and Pourbagher, R (2020). The effects of pre-treatment and

post-treatment of thymol against tert-butyl hydroperoxide (t-BHP) cytotoxicity in MCF-7 cell line and fibroblast derived foreskin. *Rep. Biochem. Mol. Biol., 9(3)*: 338-347. DOI: 10.29252/rbmb.9.3.338.

Dastanpour, S; Beitollahi, J M and Saber, K (2015). The effect of low-level laser therapy on human leukemic cells. *J. Lasers Med. Sci., 6(2)*: 74-79.

Diniz, I M; Souto, G R; Freitas, I D; de Arruda, J A A; da Silva, J M; Silva, T A and Mesquita, R A (2020). Photobiomodulation enhances cisplatin cytotoxicity in a culture model with oral cell lineages. *Photochem. Photobiol., 96(1)*: 182-190. DOI: 10.1111/php.13152.

Godswill, N N; Frank, N E G; Walter, A N; Edson, M Y J; Kingsley, T M; Arondel, V and Emmanuel, Y (2016). Oil palm: *Breeding Oilseed Crops for Sustainable Production* (Gupta, S K ed.). Academic Press, San Diego. p. 217-273.

Hattori, N; Yamada, S; Torii, K; Takeda, S; Nakamura, K; Tanaka, H and Nakayama, G (2015). Effectiveness of plasma treatment on pancreatic cancer cells. *Int. J. Oncol., 47(5)*: 1655-1662. DOI: 10.3892/ijo.2015.3149.

Ibrahim, N E S; Morsy, H and Abdelgwad, M (2021). The comparative effect of nisin and thioridazine as potential anticancer agents on hepatocellular carcinoma. *Rep. Biochem. Mol. Biol., 9(4)*: 452-462. DOI: 10.52547/rbmb.9.4.452.

Ji, X; Usman, A; Razalli, N H; Sambanthamurthi, R and Gupta, S V (2015). Oil palm phenolics (OPP) inhibit pancreatic cancer cell proliferation via suppression of NF-κB pathway. *Anticancer Res., 35(1)*: 97-106.

Ji, X; Wang, Z; Geamanu, A; Goja, A; Sarkar, F H and Gupta, S V (2012). Delta-tocotrienol suppresses Notch-1 pathway by upregulating miR-34a in nonsmall cell lung cancer cells. *Int. J. Cancer, 131(11)*: 2668-2677. DOI: 10.1002/ijc.27549.

Kara, C and Orbak, R (2009). Comparative evaluation of Nd: YAG laser and fluoride varnish for the treatment of dentinal hypersensitivity. *J. Endod., 35(7)*: 971-974. DOI: 10.1016/j.joen.2009.04.004.

Komarasamy, T V and Sekaran, S D (2012). The anti-proliferative effects of a palm oil-derived product and its mode of actions in human malignant melanoma MeWo cells. *J Oleo Sci., 61(4)*: 227-239. DOI: 10.5650/jos.61.227.

Kumar, K S; Raghavan, M; Hieber, K; Ege, C; Mog, S; Parra, N and Toles, R (2006). Preferential radiation sensitization of prostate cancer in nude mice by nutraceutical antioxidant γ-tocotrienol. *Life Sci., 78(18)*: 2099-2104. DOI: 10.1016/j.lfs.2005.12.005.

Lee, Y; Kim, H; Hong, N; Ahn, J C and Kang, H W (2020). Combined treatment of low-level laser therapy and phloroglucinol for inhibition of fibrosis. *Lasers Surg. Med., 52(3)*: 276-285. DOI: 10.1002/lsm.23131.

Loganathan, R; Selvaduray, K R; Nesaretnam, K and Radhakrishnan, A K (2021). Palm tocotrienols cause cleavage of poly-(adp)-ribose polymerase enzyme and down-regulation of cyclooxygenase-2 protein level in human breast cancer cells. *J. Oil Palm Res., 33(1)*: 119-128. DOI: 10.21894/jopr.2020.0082.

Loganathan, R; Subramaniam, K M; Radhakrishnan, A K; Choo, Y M and Teng, K T (2017). Health-promoting effects of red palm oil: Evidence from animal and human studies. *Nutr. Rev., 75(2)*: 98-113. DOI: 10.1093/nutrit/nuw054.

Marasini, R and Aryal, S (2022). Indocyanine-type infrared-820 encapsulated polymeric nanoparticle-assisted photothermal therapy of cancer. *ACS Omega, 7(14)*: 12056-12065. DOI: 10.1021/acsomega.2c00306.

Nakamura, H and Takada, K (2021). Reactive oxygen species in cancer: Current findings and future directions. *Cancer Sci., 112(10)*: 3945–3952. DOI: 10.1111/cas.15068.

Owoyele, B V and Owolabi, G O (2014). Traditional oil palm (*Elaeis guineensis* Jacq.) and its medicinal uses: A review. *TANG, 4(3)*: 16.11-16.18. DOI: 10.5667/tang.2014.0004.

Parveez, G K A; Tarmizi, A H A; Sundram, S; Loh, S K; Ong-Abdullah, M; Palam, K D P, and Idris, Z (2021). Oil palm economic performance in Malaysia and R&D progress in 2020. *J. Oil Palm Res., 33*(2): 181-214. DOI: 10.21894/jopr.2021.0026.

Pashah, Z; Hekmat, A and Hesami Tackallou, S (2019). Structural effects of diamond nanoparticles and paclitaxel combination on calf thymus DNA. *Nucleosides Nucleotides Nucleic Acids., 38(4)*: 249-278. DOI: 10.1080/15257770.2018.1515440.

Perillo, B; Di Donato, M; Pezone, A; Di Zazzo, E; Giovannelli, P; Galasso, G and Migliaccio, A (2020). ROS in cancer therapy: The bright side of the moon. *Exp. Mol. Med., 52(2)*: 192-203. DOI: 10.1038/s12276-020-0384-2.

Rahman, S N S A; Wahab, N A and Abd Malek, S N (2013). *In vitro* morphological assessment of apoptosis induced by antiproliferative constituents from the rhizomes of *Curcuma zedoaria*. *Evid.*

Based Complement. Alternat. Med., 2013: 1-14. DOI: 10.1155/2013/257108.

Razzaghi, M R; Ghazimoradi, M H; Afzali, S; Kamani, E; Mohajerani, E; Shirkavand, A and Farivar, S (2021). Effect of a low-level laser on liposomal doxorubicin efficacy in a melanoma cell line. *J. Lasers Med. Sci., 12:* 1-5. DOI: 10.34172/jlms.2021.28.

Robey, R W; Pluchino, K M; Hall, M D; Fojo, A T; Bates, S E and Gottesman, M M (2018). Revisiting the role of efflux pumps in multidrug-resistant cancer. *Nat. Rev. Cancer, 18(7)*: 452-464. DOI: 10.1038/s41568-018-0005-8.

Rola, P; Włodarczak, S; Lesiak, M; Doroszko, A and Włodarczak, A (2022). Changes in cell biology under the influence of low-level laser therapy. *Photonics, 9(7)*: 502. DOI: 10.3390/photonics9070502.

Sambanthamurthi, R; Tan, Y; Sundram, K; Hayes, K C; Abeywardena, M; Leow, S S and Sinskey, A J (2011). Positive outcomes of oil palm phenolics on degenerative diseases in animal models. *Br. J. Nutr., 106(11)*: 1664-1675. DOI: 10.1017/S0007114511002133.

Sekaran, S D; Leow, S S; Abobaker, N; Tee, K K; Sundram, K; Sambanthamurthi, R and Wahid, M B (2010). Effects of oil palm phenolics on tumor cells *in vitro* and *in vivo*. *Afr. J. Food Sci., 4(8)*: 495-502. DOI: 10.5897/AJFS.9000113.

Seragel-Deen, F; Ghani, S A A; Baghdadi, H M and Saafan, A M (2020). Combined cisplatin treatment and photobiomodulation at high fluence induces cytochrome c release and cytomorphologic alterations in HEp-2 cells. *Open Access Maced. J. Med. Sci., 8(A)*: 366-373. DOI: 10.3889/oamjms.2020.4561.

Tam, S Y; Tam, V C; Ramkumar, S; Khaw, M L; Law, H K and Lee, S W (2020). Review on the cellular mechanisms of low-level laser therapy use in oncology. *Front. Oncol., 10*: 1255. DOI: 10.3389/fonc.2020.01255.

Yan, D; Cui, H; Zhu, W; Nourmohammadi, N; Milberg, J; Zhang, L G and Keidar, M (2017). The specific vulnerabilities of cancer cells to the cold atmospheric plasma-stimulated solutions. *Sci Rep., 7(1)*: 1-12. DOI: 10.1038/s41598-017-04770-x.