The effect of near-infrared rays on the decrease in the number of adipocyte cells using the flow cytometry method

Emillia Devi Dwi Rianti, Fuad Ama

Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia

Abstract

Adipose tissue, composed of adipocyte cells, can become problematic due to an increase in cell number (hyperplasia) and size (hypertrophy). To address this concern, interventions are needed to reduce these probabilities. Near Infrared (NIR) is an electromagnetic wave that affects tissues by being absorbed and penetrating them. NIR can induce cell death (necrosis or apoptosis), where cells respond to external factors causing tissue damage. The purpose of this study was to examine the effect of near-infrared rays on the decrease in the number of adipocyte cells using the flow cytometry method. This study, conducted in vitro using adipocyte cell cultures isolated from rat visceral adipose tissue, explores the effects of NIR exposure at various distances (1 cm, 2 cm, and 3 cm) for 20 minutes. Adipocyte cells were stained and examined using Confocal laser scanning microscopy (CLSM), and the percentage of cells was determined by flow cytometry. Statistical analyses were performed using One Way ANOVA and Duncan test. Significant differences ($p<0.05$) were observed, and the Duncan test revealed variations in the percentage of living cells among control and treatment groups. The data indicated that exposure to high NIR energy with low wavelengths penetrated adipocyte cells, leading to a decrease in the percentage of cells and notable changes in cell morphology. Flow cytometry results demonstrated differences in live cell percentages, with group 2 (exposed at 2 cm for 20 minutes) being more effective, showing lower percentages of live cells. This research suggests that NIR exposure has an impact on adipocyte cell cultures, emphasizing its potential in influencing adipocyte cell behavior.

Correspondence: Emillia Devi Dwi Rianti, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia.
E-mail: emilia@uwks.ac.id

Key words: adipocyte cells, apoptosis, flow cytometry, health, near infrared rays, wavelength.

Contributions: EDDR, conceptualization, data curation, formal analysis, methodology, validation, visualization, writing – original draft, review & editing; FA, conceptualization, investigation, methodology, validation, and writing – original draft, review & editing.

Conflict of interest: the authors declare no conflict of interest.

Ethics approval and consent to participate: this research has obtained approval from the Research Ethics Commission of the Faculty of Medicine, Wijaya Kusuma University, Surabaya, with the number 59/SLE/FK/UWKS/2019.

Patient consent for publication: none.

Funding: this research did not receive external funding.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Acknowledgement: we would like to extend our thanks to Mrs. Heni Endrawati, SSi, for her help in the laboratory experiments.

Received: 14 September 2023.
Accepted: 4 December 2023.
Early access: 18 January 2024.

Healthcare in Low-resource Settings 2024; 12:11803
doi:10.4081/hls.2024.11803

Introduction

Adipocyte cells, also known as lipocytes and fat cells, are the primary cells that constitute adipose tissue, specializing in storing energy in the form of fat.[1] This tissue is situated beneath the skin and surrounding internal organs,[2] playing biochemical roles associated with the mechanisms of obesity.[3] The increase in the number of adipocyte cells is termed hyperplasia, while the enlargement of adipocyte cells is referred to as hypertrophy.[4]

Obesity poses a significant challenge and is recognized as a risk factor for various degenerative conditions.[5,6] Strategies to regulate calorie intake involve reducing energy consumption and increasing physical activity. In addition to dietary adjustments, exercise can aid individuals with obesity in sculpting a firmer physique.[7] There are three primary approaches to treating obesity: lifestyle modification (which includes adjusting diet, increasing physical activity, and behavioral therapy), pharmacotherapy (medication), and plastic surgery.[8] However, the success rates of these methods in reducing obesity have not reached their maximum potential, necessitating the exploration of alternative measures. One recommended alternative therapy for obesity involves the use of infrared technology.[9,10] This therapy utilizes irradiation from near-infrared (NIR) rays to address obesity cases.[11]

Infrared rays have the capability to enhance mitochondrial metabolism[12] and generate heat radiation at a lower frequency. Near-infrared (NIR) can impact tissues due to its extremely limited absorption range,[13] with wavelengths between 800 nm and 970 nm suggested for therapeutic purposes.[14] The energy from NIR rays is absorbed by the skin's surface layer and partially by tissue before penetrating deep into the tissue.[11] NIR constitutes a form of biological radiation that manifests within cells, leading to cell death – either necrosis or apoptosis – in both the short and long term.[15]

Apoptosis involves different mechanisms within cells and can

occur through the mitochondrial pathway.[16] It is a programmed cell death resulting from conditions inside the cell itself, such as Deoxyribonucleic acid (DNA) damage, or external stimulation. Apoptosis is a normal occurrence in various physiological processes aimed at maintaining homeostasis. Organisms utilize apoptosis to eliminate cells that are no longer necessary or useful to the body.[17] The mitochondrial pathway is a prominent route through which apoptosis can be observed, with mitochondria playing a crucial role in cell death and functioning as crosstalk organelles.[18] These organelles are pivotal in various apoptotic pathways, which are categorized into two types: dependent and independent caspases.[19] Apoptosis signaling in the dependent caspase pathway can occur both intracellularly and extracellularly. Extrinsic (extracellular) pathways are initiated by the stimulation of death receptors, while intrinsic pathways are triggered by the release of signaling factors from mitochondria within cells.[20] Therefore, this study aimed to investigate the impact of Near-Infrared (NIR) on reducing the number of adipocyte cells using the Flow Cytometry method.

Materials and Methods

The research employed a true experimental post-test-only control group design. Samples for both the experimental and control groups were selected through randomization. Primary cells were generated using adipocytes extracted from 4-week-old Wistar Rattus Norvegicus strain mice, totaling 20 samples. The adipocyte cells were cultured by isolating them from visceral adipose tissue, and the research was conducted in vitro on these adipocyte cells. Adipocyte cell cultures were subjected to NIR light for a 20-minute treatment. Treatment 1 involved NIR exposure at a distance of 1 cm, treatment 2 at a distance of 2 cm, and treatment 3 at a distance of 3 cm. The variables comprised the dependent variable, which is the apoptosis of adipocyte culture cells, and the independent variable, which is NIR, with an exposure time of 20 minutes and distances of one, two, and three cm. This research has obtained approval from the Research Ethics Commission of the Faculty of Medicine, Wijaya Kusuma University, Surabaya, with the number 59/SLE/FK/UWKS/2019. The research and treatment of adipocyte cell culture were conducted at the Parasite Laboratory, Brawijaya University, Malang. Observations of adipocyte cells were carried out in the laboratory of the Center for Biological Sciences, Brawijaya University, Malang.

Light-Emitting Diode (LED) Near-Infrared (NIR)

The infrared ray was based on the wavelength of the NIR LED calibrated at the Applied Electroelectronics and Electromagnetic Laboratory, where the energy produced was calculated. The wavelength had an output spectrum ranging from about 910 nm to 1010 nm, with the infrared LED power being approximately 1 mW and a diameter of 5 mm. NIR LEDs were exposed to adipocyte cell cultures at distances of 1 cm, 2 cm, and 3 cm for a duration of 20 minutes.

Adipocyte cell culture

The adipose tissue cells were washed with 70% ethanol, isolated, and placed in a 50 mL Falcon tube containing Dulbecco's Modified Eagle Medium (DMEM). The Falcon, containing 3 mL of DMEM medium to which type II collagenase had been added, was then incubated in a 37 °C water bath shaker for 20 minutes and centrifuged at 1500 rpm for 7 minutes. The supernatant was discarded, and the pellet was mixed with 3-6 mL of DMEM containing 10% Fetal Calf Serum (FCS) and 1/100 pen-strep, filtered, and then transferred to a culture flask. It was stored in a CO_2 incubator for 2 hours, the medium was changed, and then put back in the CO_2 incubator.[21]

Adipocyte morphology examination with Confocal Laser Scanning Microscopy (CLSM)

The observation of adipocyte morphology was conducted using the Oil Red staining method. Cells from each treatment were prepared on a cover slide after being washed with PBS. Subsequently, the cells were fixed with 4% formaldehyde for 30 minutes and washed twice with PBS (300 µL per well). They were then stained with Red Oil in a 3:2 ratio with sterile water for 30 minutes. The cells were incubated until dry and subsequently observed under an Olympus light microscope at 400x magnification. Additionally, cell observation can be conducted using the CLSM technique, an optical imaging method that enhances optical resolution and contrast in micrographs by utilizing a spatial pinhole to block out-of-focus light.[22]

Percentage of adipocyte cell apoptosis with flow cytometry

After each tube underwent apoptotic treatment, it was centrifuged at 2000 rpm for 3 minutes, and the cells were prepared for the Flow Cytometry test. The media in the tube was discarded, and 100 µL of Annexin V-PI reagent was added to the tube, followed by the addition of 350 µL of buffer. The contents were mixed and homogenized using a vortex. Subsequently, the mixture was incubated at room temperature in a dark place for 10 minutes. The cell suspension was then transferred to a flow cytometry tube, and it was ready to be injected into a flow cytometry tool. The percentage of cells was measured using Flow Cytometry and analyzed using the Cell-Quest software (Becton Dickinson FACS Calibur).[23]

Statistical analysis

The data obtained from the calculation of energy from the wavelength of the NIR LEDs and Flow Cytometry analysis measurements for each treatment were expressed as mean ± SD. The data underwent analysis using One-Way ANOVA at a confidence level of $\alpha = 0.05$ to determine significant results. Subsequently, Duncan's test was employed to identify differences between groups.

Results

The flow cytometry method is a laboratory technique capable of differentiating apoptotic and necrotic cells. This method is widely employed in modern biomedical research and clinical applications. Flow cytometry involves the rapid analysis of single cells or particles suspended in a buffered salt-based solution as they flow through a single or dual laser. It provides rapid, quantitative, and reliable imaging for the detection of apoptosis. The data results from flow cytometry are presented in Table 1.

Flow cytometry

Observations on apoptosis were conducted using the flow cytometry method, which functions to rapidly count live cells, necrosis, and apoptotic cells. Tests utilized Annexin V proteins specifically related to the phosphatidylserine present in the cell plasma membrane during the apoptotic process. Observations were made after giving treatment for 1 hour and then analyzed. Figure 1 displays the results of the Duncan test. The Lower Right (LR) results demonstrate differences between treatment groups H and

groups 1, 2, and 3. Light is a form of electromagnetic radiation that exhibits both wave and particle properties. It is characterized by its energy content, and when light penetrates a surface, some of it is reflected. Light's nature involves energy penetrating the surface and refracting perpendicular to the surface, with its particles being photons. Energy is the capacity or effort to move electrons from one path to another, measured in Joules. The amount of energy each light wave possesses when approaching the sensor surface can be calculated using the equation $E = hc/\lambda$, where E is the absorbed energy (J), h is Planck's constant (6.626 x 10-34 Joules/sec), and c is the speed of light (3 x 108 m/sec).

Data for different tests were calculated using SPSS software version 20.0. Significant differences between averages were analyzed using one-way analysis of variance (ANOVA). The results for energy and wavelength indicated significant differences (sig. 0.000 < 0.05). The Duncan test was employed to identify differences between groups, such as E7 with E6, which had nearly the same average values of 0.197 and 0.198. On the other hand, groups E7 with E1 showed the most significant difference in average values, with 0.197 and 0.209.

Energy is the fundamental characteristic of light, allowing it to penetrate surfaces. The results of the statistical analysis indicated a significant difference with a p-value < 0.05 for the energy values within the NIR wavelength range of 910 nm-1010 nm. The energy associated with lower wavelengths, indicative of greater surface-penetrating power, exhibited higher energy values (Figure 2).

Morphology of adipocyte cell culture

Fat droplets in adipose tissue can be unilocular and/or multilocular. A unilocular cell features a large lipid droplet, pushing the cell nucleus toward the plasma membrane, resulting in a ring-like appearance. Unilocular cells are characteristic of white fat tissue and vary in size from 20 to 200 microns. Mitochondria are primarily located in the periphery of the cell, where the thicker cytoplasm is in proximity to the cell nucleus. Large fat droplet cells lack small intracellular organelles.

Morphological examination of adipocyte cell culture was conducted using Oil Red staining to identify the presence of lipid droplets. Fat cells serve as repositories for energy reserves in the form of triacylglycerol, which manifests as lipid droplets. Adipocyte cell droplets, revealed through Oil Red O staining,

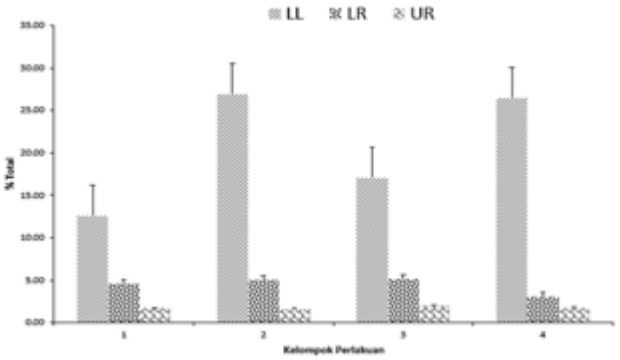

Figure 1. Detection and measurement of adipocyte cell characteristics using the flow cytometry method (1= group H as control group; 2= treatment group 1; 3= treatment group 2; 4= treatment group 3; with LL: live cells, LR: early apoptosis, UR: late apoptosis/dead).

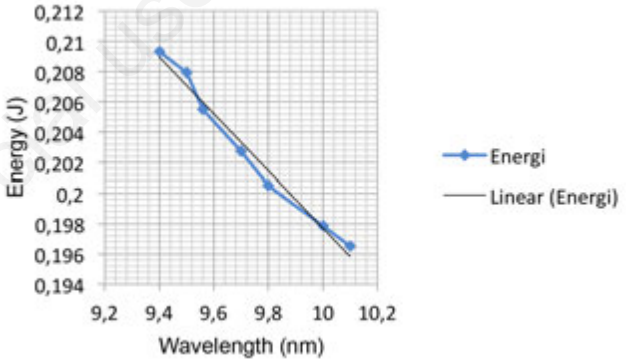

Figure 2. The results of energy and wavelengths with an output spectrum of around 910 nm -1010 nm.

Table 1. Measurement of adipocyte cell characteristics using the flow cytometry method.

No	LL Lower Left (bottom left) %	LR Lower Right (bottom right) %
1.1	41.1	2.32
1.2	37.19	2.42
1.3	26.60	4.22
1.4	27.38	5.86
2.1	36.21	4.61
2.2	34.55	4.27
2.3	14.29	5.67
2.4	19.92	4.55
3.1	35.81	2.66
3.2	32.90	2.76
3.3	25.73	3.36
3.4	27.33	2.76

appeared red. The highest resolution images of adipocyte cells were obtained using CLSM (Confocal Laser Scanning Microscopy) (Figure 3).

Discussion

Energy is a beam of light in the form of an electric-magnetic field generated by electromagnetic waves and concentrated in the form of small packages called photons. Light, when considered as a flow of particles, is called a photon, each carrying a specific amount of energy per photon.[24,25] Higher energy values result in a more substantial response and are beneficial for observing effects in deeper tissues.[26] Infrared radiation carries energy that can penetrate body tissues. Additionally, NIR exposure has the capability to penetrate subcutaneous tissue. Near-Infrared (NIR) irradiation induces both thermal and non-thermal heating, stimulating collagen and elastin production, and is utilized as a method of cell interference.[12]

The calibration results of NIR LEDs with a wavelength ranging from 910 nm to 1010 nm impact the target and depth. Wavelengths below 1100 nm can directly affect target selection and depth and are also absorbed by melamine in the surface layer of the skin.[25,27] The NIR wavelength of 980 nm can penetrate tissues to varying depths depending on the wavelength, time, and tissue.[28] NIR can stimulate the biological functions of cells located in the stomach area. The basic mechanism of NIR, with its low intensity, shows a synergistic effect in reducing fat. NIR rays can be utilized as effective methods for fat-reducing therapy.[29]

The results of exposure to NIR rays revealed a significant effect on morphological changes in adipocyte cell cultures stained with Oil Red O. Exposure to NIRs with high energy values and low wavelengths can penetrate adipocyte cells, as evidenced by morphological changes resulting in a reduced number of adipocyte cells.[30] Normal adipocyte cells typically exhibit a round shape with clear cell nuclei in the peripheral part of the cell, smooth cell membranes, and the presence of large pleated droplets almost filling the cell portion (Figure 3). Adipocyte cells undergoing morphological changes can be characterized by an enlarged adipocyte cell and an uneven cell membrane.

The results from flow cytometry of adipocyte cells (Figure 3) illustrate cells undergoing apoptosis and the relative distribution of cell populations. The measurement of cell population distribution, seen through light, enhances communication between cells and stimulates chromophores (enzymes sensitive to light) involved in various metabolic processes. Figure 3's flow cytometry results indicate that the mean LL (Lower Left; representing the percentage of living cells) with the percentage of living cells from the different H treatments (control group) differed from groups 1, 2, and 3. The mean LR represents the percentage of cells undergoing initial apoptosis, showing a difference between the control group H and groups 1, 2, and 3. The mean LR (Lower Right; showing the percentage of cells undergoing apoptosis) indicates that the percentage of cells undergoing initial apoptosis has no significant difference between groups H and groups 1, 2, and 3 due to the initial apoptosis treatment in adipocyte cell cultures. Apoptosis is a programmed cell death process where the cell nucleus solidifies, the cell size shrinks, and the cell bodies undergo changes.[31]

Apoptosis is triggered by signaling pathways and regulated by complex extrinsic and intrinsic ligands. It is a programmed cell death that occurs due to conditions within the cell itself or external stimulation. Under normal conditions, apoptosis is part of various physiological processes to maintain homeostasis, meaning it is utilized by organisms to eliminate cells that are no longer needed by the body.[17] Apoptosis occurs through an intrinsic pathway, initiated by changes in the mitochondrial membrane potential, leading to the secretion of cytochrome-c from the mitochondria into the cytoplasm.

The relationship between radiation, apoptosis, and clonogenic cell survival is complex because, after radiation, cells can either undergo apoptosis during mitosis, survive, or die without mitosis.[32] Radiation induces apoptosis by altering the mitochondria, followed by the activation of the caspase enzyme. The process of

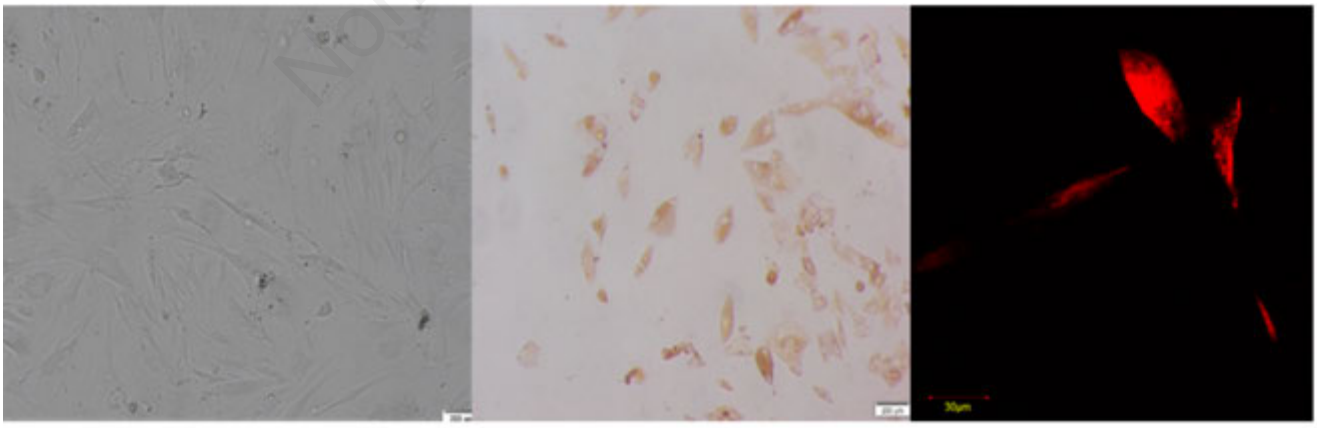

Figure 3. The results of the morphology of adipocyte cell culture, with conditions after treatment and administration of Oil Red O and CLSM. a) adipocyte cell culture before treatment (40x), b) adipocyte cells after treatment and oiled o (100x), c) adipocyte cells after treatment then observed with CLSM (600x).

apoptosis can commence in the mitochondria, either through DNA damage or as a response to the cell membrane.[33] Apoptosis can be triggered by increasing energy in the cell membrane, involving the activation of ceramide through the hydrolysis of sphingomyelin to ceramide by the acid sphingomyelinase enzyme. Ceramide serves as a second method to activate apoptosis through BAX (Bcl-2 Associated x protein) stimulation. This stimulation leads to the binding of BAX to the outer membrane of the mitochondria, causing the release of cytochrome-c and activation of caspase.[33,34] The BAX molecule located in the outer mitochondrial membrane functions as a molecule that induces apoptosis by triggering the release of cytochrome c. Subsequently, cytochrome c binds with Apaf-1, ATP, and caspase-9, activating procaspase-3 to become caspase-3, which then cleaves the cytoskeleton.[33,34]

Conclusions

Exposure to high NIR energy and low wavelengths can penetrate adipocyte cells, resulting in a decrease in adipocyte cell count, as demonstrated by the flow cytometry method. Additionally, there is a reduction in the morphology of adipocyte cells. The flow cytometry method is employed to rapidly count live cells, cell necrosis, and apoptosis. The results indicate that NIR influences adipocyte cell culture, revealing a difference in the percentage of live cells between the control group and groups 1, 2, and 3. Notably, Group 2 exhibits a lower percentage of live cells. Consequently, exposure at a distance of 2 cm for 20 minutes proves to be more effective.

References

1. Birbrair A, Zhang T, Wang ZM, et al. Role of pericytes in skeletal muscle regeneration and fat accumulation. Stem Cells Dev 2013;22:2298–314.
2. Chait A, den Hartigh LJ. Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. Front Cardiovasc Med 2020;7:22.
3. Longo M, Zatterale F, Naderi J, et al. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. Int J Mol Sci 2019;20(9).
4. Stenkula KG, Erlanson-Albertsson C. Adipose cell size: importance in health and disease. Am J Physiol Integr Comp Physiol 2018;315:R284–95.
5. Mahiroh H, Astutik E, Pratama RA. The Association of Body Mass Index, Physical Activity and Hypertension in Indonesia. J Ners 2019;14:16–22.
6. Rizal A, Hermanto TJ, Murtiwi S, Ernawati. A comparison of HBA1c in pregestational diabetes patients with obesity. Biochem Cell Arch 2019;4745–8.
7. Rejeki PS, Baskara PG, Herawati L, et al. Moderate-intensity exercise decreases the circulating level of betatrophin and its correlation among markers of obesity in women. J Basic Clin Physiol Pharmacol 2022;33:769–77.
8. Piyakong D, Apiratanawong S, Suasing C. Insights from leaders on effectively addressing overweight and obesity in the Thai community. J Ners 2023;18:117–23.
9. Gao M Jiao, Xue H Zhong, Cai R, et al. A Preliminary Study on Infrared Thermograph of Metabolic Syndrome. Front Endocrinol 2022;13:851369
10. Kim S, Kim Y, Lee G, Kim J. Does treadmill walking with near-infrared light applied to the abdominal area reduce local adiposity and body weight? J Phys Ther Sci 2017;29:1753–6.
11. Gholami L, Afshar S, Arkian A, et al. NIR irradiation of human buccal fat pad adipose stem cells and its effect on TRP ion channels. Lasers Med Sci 2022;37:3681–92.
12. Averbeck D, Rodriguez-Lafrasse C. Role of Mitochondria in Radiation Responses: Epigenetic, Metabolic, and Signaling Impacts. Int J Mol Sci 2021;22.
13. Liu TM, Conde J, Lipiński T, Bednarkiewicz A, Huang CC. Revisiting the classification of NIR-absorbing/emitting nanomaterials for in vivo bioapplications. NPG Asia Mater 2016;8:e295–e295.
14. Larkin-Kaiser KA, Christou E, Tillman M, et al. Near-infrared light therapy to attenuate strength loss after strenuous resistance exercise. J Athl Train 2015;50:45–50.
15. Nakajima K, Takakura H, Shimizu Y, Ogawa M. Changes in plasma membrane damage inducing cell death after treatment with near-infrared photoimmunotherapy. Cancer Sci 2018;109:2889–96.
16. Fakhlaei R, Selamat J, Khatib A, et al. The toxic impact of honey adulteration: A review. Foods 2020;9(11).
17. Orrenius S, Nicotera P, Zhivotovsky B. Cell Death Mechanisms and Their Implications in Toxicology. Toxicol Sci 2011;119:3–19.
18. Luqman EM, Widjiati, Mafruchati M, et al. Crosstalk between necrosis and apoptosis of embryonal cerebral cortex neuron mice (Mus musculus) caused by carbofuran exposure. Res J Pharm Technol 2019;12:5492–8.
19. Ahmad MA, Aung YY, Widati AA, et al. A Perspective on Using Organic Molecules Composing Carbon Dots for Cancer Treatment. Nanotheranostics 2023;7:187–201.
20. Peng F, Liao M, Qin R, et al. Regulated cell death (RCD) in cancer: key pathways and targeted therapies. Signal Transduct Target Ther 2022;7:286.
21. Lin J, Della-Fera MA, Baile CA. Green tea polyphenol epigallocatechin gallate inhibits adipogenesis and induces apoptosis in 3T3-L1 adipocytes. Obes Res. 2005 Jun;13(6):982–90.
22. Elliott AD. Confocal Microscopy: Principles and Modern Practices. Curr Protoc Cytom 2020;92:e68.
23. Endharti AT, Wahyuningtyas TE, Handono K, et al. Dendrophthoe pentandra leaves extract promotes apoptotic effects of doxorubicin in human breast cancer cell via modulation of intracellular calcium and survivin. J Appl Pharm Sci 2018;8:39–43.
24. Zhang Y, Feng Y, Ming X, Deng J. Energy Modulated Photon Radiotherapy: A Monte Carlo Feasibility Study. Biomed Res Int 2016;2016:7319843.
25. Chang DS, Lasley FD, Das IJ, et al. Characteristics of Photon Beams. In: Chang DS, Lasley FD, Das IJ, Mendonca MS, Dynlacht JR, editors. Basic Radiother Physics Biol 2021:67–73.
26. Samad FA, Mohamed T. Intensity and wavelength-dependent two-photon absorption and its saturation in ITO film. Appl Phys A Mater Sci Process 2023;129(1).
27. Tanaka Y. Impact of near-infrared radiation in dermatology. World J Dermatol 2012;1:30–7.
28. Henderson TA, Morries LD. Near-infrared photonic energy penetration: can infrared phototherapy effectively reach the human brain? Neuropsychiatr Dis Treat 2015;11:2191–208.
29. Cristiano L. Use of infrared-based devices in aesthetic medicine and for beauty and wellness treatments. Infrared Phys Technol 2019;102:102991.

30. Onyancha RB, Ukhurebor KE, Aigbe UO, et al. A review of the capabilities of carbon dots for the treatment and diagnosis of cancer-related diseases. J Drug Deliv Sci Technol 2022;78.
31. Zhang Y, Chen X, Gueydan C, Han J. Plasma membrane changes during programmed cell deaths. Cell Res 2018;28:9–21.
32. Sia J, Szmyd R, Hau E, Gee HE. Molecular Mechanisms of Radiation- Induced Cancer Cell Death: A Primer. Front Cell Develop Biol 2020;8.
33. Häcker G, Haimovici A. Sub-lethal signals in the mitochondrial apoptosis apparatus: pernicious by-product or physiological event? Cell Death Differ 2023;30:250–7.
34. Patwardhan GA, Beverly LJ, Siskind LJ. Sphingolipids and mitochondrial apoptosis. J Bioenerg Biomembr 2016;48:153–68.