https://doi.org/10.31925/farmacia.2025.2.7 **ORIGINAL ARTICLE**

THE INFLUENCE OF *MORINGA OLEIFERA* OIL ON NEUROBLASTOMA BASAL OXIDATIVE STRESS, INFLAMMATION AND AUTOPHAGY MECHANISM

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Abstract

Despite significant medical advances in neuroblastoma (NB) treatment, therapy resistance is a significant barrier to access to curative cancer treatments. This study investigates the effects of different concentrations of *M. oleifera* oil on drug resistance and reaction, basal oxidative stress in the SH-SY5Y neuroblastoma cell line, on one hand, and autophagy as a mechanism for tumour and cell survival on the other. First, we profiled the fatty acid composition of seed oils from *M. oleifera* using gas chromatography–mass spectrometry (GC–MS). Using different concentrations of *Moringa olivera* oil (50 - 200 µg/mL), the MTT cell proliferation assay was employed to assess cell survival in SH-SY5Y cell lines. Specific antioxidants and proinflammatory markers were estimated; LC3 as a specific marker for autophagy was evaluated, using a flow cytometer. LC3 expression was significantly higher in the SH-SY5Y-treated cell line at the highest concentration (200 µg/mL) compared to the control. A status of dose and time independent reductions in pro-inflammatory cytokines and free radicals as markers of neuroinflammation and oxidative stress were recorded. *M. oleifera* oil could be used to reduce SH-SY5Y drug resistance initially by antioxidant and anti-inflammatory properties, rather than the essential role of autophagy, which is a novel finding as it shifts focus from autophagy as the central mechanism of action.

Rezumat

Deși s-au realizat progrese semnificative în tratamentul neuroblastomului, rezistența la terapie rămâne un obstacol major pentru dezvoltarea unor tratamente eficiente. Acest studiu a investigat efectele diferitelor concentrații de ulei de *Moringa oleifera* asupra rezistenței la medicamente, a stresului oxidativ bazal și a autofagiei ca mecanism de supraviețuire tumorală și celulară. S-a analizat compoziția în acizi grași a uleiurilor din semințele de *M. oleifera* utilizând cromatografia de gaze cuplată cu spectrometrie de masă (GC–MS). Ulterior, utilizând concentrații diferite de ulei de *M. oleifera* (50 - 200 µg/mL), a fost evaluată supraviețuirea celulară în linia SH-SY5Y prin testul MTT de proliferare celulară. De asemenea, au fost determinate nivelurile unor markeri specifici ai stresului oxidativ și inflamației, iar expresia LC3, marker al autofagiei, a fost analizată prin citometrie în flux. Rezultatele au arătat o creștere semnificativă a expresiei LC3 în celulele tratate cu concentrația maximă (200 µg/mL) comparativ cu grupul martor. În plus, s-au observat reduceri ale citokinelor proinflamatorii și ale radicalilor liberi, markeri ai neuroinflamației și stresului oxidativ, într-un mod independent de doză și timp. Astfel, uleiul de *M. oleifera* ar putea reduce inițial rezistența la medicamente în linia celulară SH-SY5Y prin proprietățile sale antioxidante și antiinflamatorii, mai mult decât prin autofagie.

Keywords: SH-SY5Y, *Moringa oleifera* oil, antioxidants, proinflammatory markers, LC3 autophagy marker

Introduction

The therapeutic strategies that focus on preventing the reactive oxygen species (ROS) formation mediated by antioxidants seem to have an impact on delaying the disease's progression. Numerous synthetic antioxidants have been established to be potent radical scavengers, but they are also mutagenic and cause cell damage. The primary cause of ROS is environmental stress. ROS is a typical consequence of the regular metabolism of oxygen molecules. Increased ROS levels, outside or inside the cells, cause severe damage to all biological macromolecules, primarily lipids, proteins, and nucleic acids. Cells produce ROS, including O^{2-} and H2O2, by reducing oxygen to O^{2-} using NADPH or NADH oxidase as electron donors. Oxidative burst occurs when O^{2-} is transformed to H_2O_2 by dismutase, which can occur spontaneously or with enzymes. H_2O_2 has significant physiological and pathological consequences because it is highly diffusible and can permeate the plasma membrane [1]. The brain is prone to producing ROS (such as O^{2-}, H_2O_2 and OH•), making neuronal cells more sensitive to oxidative stress than cells in

other tissues. Oxidative stress accumulation is thought to be the primary cause of neurodegenerative diseases. Furthermore, oxidative stress, by triggering mitochondrial malfunction, causes apoptotic neuronal cell death, which leads to neurodegenerative illnesses like Alzheimer's and Parkinson's. Building on that fact, inhibiting oxidative stress is considered an energetic technique to avoid neuronal advanced disease [2]. Thus far, no effective medications that entirely prevent neuronal cell death in neurodegenerative disorders are available [3]. Therefore, efforts have been made in discovering new strategies to combat oxidative stress using antioxidants derived from various sources, both synthetic and natural. Several plant species from different parts of the world have been discovered to be rich suppliers of bioactive chemicals with potential medical advantages. *M. oleifera*, a cold and drought-resistant plant of the monogenetic family *Moringaceae*, is distinguished by its varied usage as a food additive and supplement therapy [4, 5].

When combined with its dietary components, such as polyphenols, flavonoids, glucosinolate and isothiocyanate, phenolic acid, alkaloids and sterols, and terpene enolic acids, the *Moringa oleifera* plant is effective for over 80 diseases as antioxidants, anti-inflammatory, immune system modulators, and anti-diabetic properties, in addition to several significant fatty acids derived from seed oil [6-8]. The seeds of *M. oleifera* are good antioxidants, able to reduce oxidative damage associated with ageing and cancer [9]. Many bioactive compounds isolated from *M. oleifera* seeds are potential antitumor promoters [10]. However, recent study results found that the ethanol extract of *M. oleifera* seeds had no significant effect in inhibiting the proliferation of breast and colorectal tumour cells. Nevertheless, a recent investigation observed a cytotoxic effect of *M. oleifera* oil in several cancer cell lines [11].

It has been reported that exogenous polyunsaturated fatty acids such as linoleic acid (LA), oleic acid (OA) and palmitic acid (PA) can temper the cytotoxic activity of anti-cancer drugs [12, 13]. High linoleic acid in olive oil significantly affects oxidative stress markers and lipid peroxidation in SH-SY5Y cells under basal conditions. Oleic acid is another mono-unsaturated fatty acid that is found in high percent in moringa oil and may considered as one of the major components of the extra virgin of olive oil [14, 15], oleic acid possesses anti-inflammatory, antioxidative characteristics, prevents cardiovascular diseases and breast cancer it has the capability of lowering hypertension and persuade good sleep, guards' bones and calms the nervous system. Oil is rich in vitamins A and E, with strong antibacterial properties and another health benefit. Moreover, PA represents another one of the moringa oil fatty acids. The tight homeostatic control of PA tissue concentration is likely related to its fundamental physiological role in several biological functions. Particularly in infants PA seems to play a crucial role as recently thoroughly revised by Innis [16]. The disruption of PA homeostatic balance, implicated in different physiological and pathological conditions, such as neurodegenerative diseases and cancer, is often related to an uncontrolled PA endogenous biosynthesis.

SHSY5Y, a neuroblastoma cell line. A triple-subcloned cell line obtained in 1970 from a bone marrow biopsy of a neuroblastoma tumour, originating from the SK-N-SH neuroblastoma cell line. This cell line provides an unlimited supply of cells of human origin with similar biochemical characteristics to human dopaminergic neurons [17]. It serves as a model for neurodegenerative disorders since the cells can be converted to various types of functional neurons by the addition of specific compounds.

Autophagy, or cellular self-digestion, is a cellular pathway involved in protein and organelle degradation, with many connections to human disease and physiology. Autophagy serves two functions in various diseases. In the early stages of tumorigenesis, autophagy plays a tumour-suppressing role by maintaining genomic integrity and inhibiting tissue damage and inflammation *via* processes involving quality control systems and oxidative stress responses [18, 19]. However, in the advanced stages of tumour development, autophagy provides nutrients to cancer cells. It promotes immune escape (*via* degradation of MHC-I on the surface of cancer cells, among other functions [20, 21]. However, many of the potent agents have failed to yield positive results in clinical trials due to the complexity of neuroblastoma in children. Consequently, the goal of the current research is to examine the antioxidant, anti-inflammatory, and autophagic response of moringa oil as a natural product against basal oxidative stress of SHSY5Y.

Materials and Methods

Chemicals and materials

Chemicals and reagents Dulbecco's Modified Eagle's Medium (DMEM) culture medium containing 4.5 g LG1 glucose was purchased from AppliChem, Darmstadt, Germany. Foetal bovine serum (FBS), 0.25% Trypsin-EDTA solution and antibiotic/ anti-mycotic solution were purchased from GIBCO® Invitrogen, Life Technologies, USA. Other chemicals and reagents were of cell culture grade and were purchased from Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA. Disposable culture ware and consumable materials were procured from Corning, New York, USA.

Preparation of Moringa seed oils

Oil samples for the current study were obtained from the National Research Centre's special unit of refining oils in Cairo, Egypt. Cold pressing was used to extract

the oils from *M. oleifera* seeds to avoid any changes in oil properties. Unless otherwise specified, an oil sample stock solution (1 mg/mL) was prepared with DMSO as the solvent. A series of different dilutions were prepared from the stock solution and used as working solutions [11]. Finally, 0.22 mm sterile syringe filters (Millipore, USA) were used to filter the working solution sets.

GC/MS analysis of Moringa oleifera oil

The GC-MS analysis of the fatty acids methyl ester was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Centre with the following specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system had a TG-WAX MS column (30 m x 0.25 mm *i.d.*, 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio 1:10 using the following temperature program: 80°C for 1 min; rising at 4ºC/min to 300°C and held for 5 min. The injector and detector were held at 240°C. 3 µL of the sample were continuously injected. Mass spectra were obtained by electron ionisation (EI) at 70 eV, using a spectral range of m/z 35 - 550. Most compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

MTT assay

The human neuroblastoma cell strain SH-SY5Y was acquired from the National Cancer Institute in Cairo, Egypt; the cells underwent digestion, suspension in DMEM medium supplemented with 10% FBS and subsequent quantification. They were then placed in a 96-well plate at a density of 1×10^4 cells *per* well. One day after inoculation, the adherent cells were shifted to serum-free DMEM and exposed to *Moringa oleifera* oil at 50, 100, 150 or 200 µM concentrations for 24 and 48 hrs. The culture media were collected and stored at -20°C for future use and the cells were reacted with 5 mg/mL G1 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT solution for 4 hrs. The reaction was terminated by adding 100 µM L DMSO and the absorbance at 570 nm was determined using an enzyme linked immunosorbent assay reader.

For cell viability assay, cells were seeded in a 96-well culture plate at a concentration of 5×10^3 cells *per* well overnight. Following overnight incubation, the cells were subjected to various conditions for 24 hrs. Their proliferation was assessed using the MTT cell proliferation assay (Cell Titer 96 Non-Radioactive Cell Proliferation Assay, Promega) as *per* the manufacturer's guidelines. The absorbance at 570 nm was measured using the Spectra Max 190 microplate spectrophotometer (Molecular Devices, Sunnyvale, California). The assays were conducted in triplicate, and the relative cell viability (expressed as a percentage of the control) was determined using the formula:

Mean OD of treated cells × 100 Mean OD of control cells at each time point

The treated cells were compared with control cells that had undergone treatment with the vehicle only.

Biochemical analysis: Glutathione (GSH) and Malondialdehyde level

The GSH level was estimated using the kit provided by Randox Company according to the manufacturer's instructions. Malondialdehyde (MDA) level, as an index of lipid peroxidation, was measured using the kit supplied by Randox Company according to the manufacturer's instructions. Superoxide oxide dismutase (SOD) level was estimated using the kit supplied by Randox Company according to the manufacturer's instructions.

Determination of IL-16 and IL-10

The IL-16 and IL-10- containing supernatants (BD Bioscience Pharmingen, Franklin Lakes, New Jersey, USA) were analysed using ELISA kits following the manufacturer's prescribed protocol. In summary, the capacity of cells to release IL-16 in response to cytokines was assessed through a sandwich ELISA. A flat-bottom 96-well microtiter plate (Greiner Bio-One, Kempten, Germany) was coated with 100 µL/well of anti-human IL-16 mAb (2 mg/mL in a mixture of sodium carbonate and sodium bicarbonate, pH 9.5) overnight at 4°C. Following washes with phosphate-buffered saline (PBS; pH 7.0) and 0.05% Tween-20, the plate was blocked with 10% foetal calf serum (FCS). The IL-16 standards (rHu IL-16) were prepared in a solution of PBS (pH 7.0) and 10% FCS using serial dilutions. Standards or supernatants (100 µL/well) were plated in triplicate and incubated at room temperature for 2 hrs. After three washes, 100 µL/well of biotinylated anti-human IL16 mAb (100 ng/mL G1 in PBS, pH 7.0 and 10% FCS) was added, followed by 100 µL/well of streptavidin–peroxidase conjugate. The chromogen substrate (100 µL/well) was applied; after 30 min, 10% H_2SO_4 was added to terminate the reaction. Absorbance was read at 450 nm using an automated microplate reader (BioTek Instruments, Richmond, California, USA). The quantification of IL-10 was also conducted *via* ELISA using a dedicated kit.

Determination of TNF

The collected supernatants from each treatment were incubated at room temperature and used to measure the TNF-α level using specific ELISA kits, according to the manufacturer's instructions. The TNF-α cytokine levels are shown as the mean ± SD (picograms of each cytokine *per* millilitres).

Flow cytometric detection of autophagy (LC3)

Detection of Autophagy marker LC3 was performed according to Detection of Autophagy marker following

the manufacturer's instructions using Accuri C6 Becton Dickinson flow cytometer. Briefly, 100 µL of cell suspension were blocked with 200 µL of protein block solution (2% BSA, cat. No. 810652; Merck KGaA) for 20 min, at room temperature. Then samples were fixed using 200 µL (1% paraformaldehyde) for 20 min, at room temperature as directed in the instructions for the IntraPrep permeabilisation reagent (cat. No. GAS003; Invitrogen; Thermo Fisher Sci., Inc.). Samples were incubated with 10 µL of primary antibody (rabbit anti LC3A/II (1:100, cat. No. 4108; Cell Signalling Technology, Inc.) was added to the sample in the dark at room temperature for 15 min and washed twice with PBS/BSA and centrifuged at 2000 rpm for 5 min. The supernatant was discarded and 10 µL of secondary polyclonal antibody (IgG) labelled with flour chrome Fluorescein Isothiocyanate (FITC) were added to the resident cells at room temperature in the dark for 15 min. Then, the cells were washed twice with PBS/BSA, as mentioned above. Finally, the labelled cells were fixed with 200 µL of 0.5% paraformaldehyde at 37°C overnight and prepared for flow cytometric analysis. The control cells were incubated with 10 µL secondary antibody at room temperature for 15 min and immediately analysed on an Accuri C6 flow cytometer (Becton Dickinson, Sunnyvale, California, USA). Histogram derived from flow cytometry was obtained with the computer program Accuri C6 software. Staining values were calculated as the percentage of the total number of cells counted. All experiments were repeated three times.

Results and Discussion

GC/MS analysis of Moringa oleifera oil

The GC/MS phyto-analysis of *Moringa oleifera* oil consists of 9 compounds. The total peak areas of the detected compounds are 100%. The probabilities of the structures of the detected compounds were listed in Table I and Figure 1. The major compounds were linoleic acid, methyl ester 39.69%, oleic methyl (28.81%), palmitic acid (14.85%), stearic acid methyl ester (7.64%), behenic acid methyl ester (2.13%), arachidic acid methyl ester (1.49%).

Table I

GC-MS Phyto analysis of *Moringa* essential oil

R.T.	Area %	Compounds
31.52	14.85	Palmitic acid, methyl ester
35.42	39.69	Linoleic acid, methyl ester
35.64	28.89	Oleic acid, methyl ester
35.72	2.53	Elaidic acid, methyl ester
36.25	7.64	Stearic acid, methyl ester
40.59	1.49	Arachidic acid methyl ester
44.61	2.13	Behenic acid methyl ester
48.32	1.34	Tetracosanoic acid, methyl ester

These compounds were identified using computer search user-generated reference libraries, incorporating mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed based on its mass spectral fragmentation. Reference compounds were co-chromatographed, when possible, to confirm GC retention times.

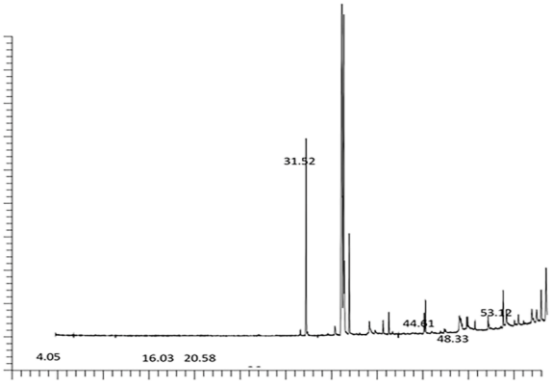

Figure 1.

GC-MS chromatograms of *M. olifera* oil. Different fatty acids in moringa seed oil

Anti-proliferative effect of Moringa oleifera

Figure 2 shows the cell viability of moringa essential oils against the SH-SY5Y neuroblastoma cell line compared to treated normal cells.

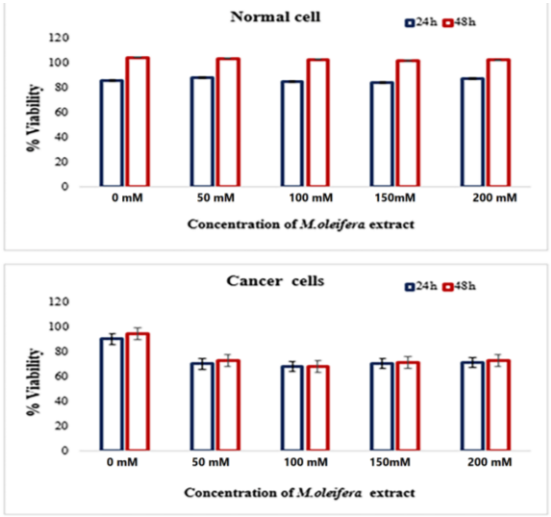

Figure 2.

Cell viability of *M. oleifera* extract against SH-SY5Y neuroblastoma cell line compared to the treated normal cells

Anti-inflammatory and antioxidant of Moringa oleifera

The anti-inflammatory and antioxidant effects of moringa oil extract on the SH-SY5Y neuroblastoma cell line compared to normal cells were shown in

Figure 3(a-d) and Figure 4(a-d). It can be easily noticed that the moringa oil exhibited dose-independent antioxidant and anti-inflammatory effects against the SH-SY5Y neuroblastoma cell line at a concentration of 200 µm. Figure 4 demonstrated much lower TNF-α, IL-10, IL-6 and IL-10 levels than untreated cells.

Detection of Autophagy marker LC3

Finding the autophagy marker using an Accuri C6 Becton Dickinson flow cytometer and the manufacturer's instructions, LC3 was performed in accordance with the detection of the autophagy marker. Figure 5(a-b) illustrates the remarkable increase of autophagy following treatment with 200 µm as the highest effective concentration of moringa essential oil compared to untreated controls.

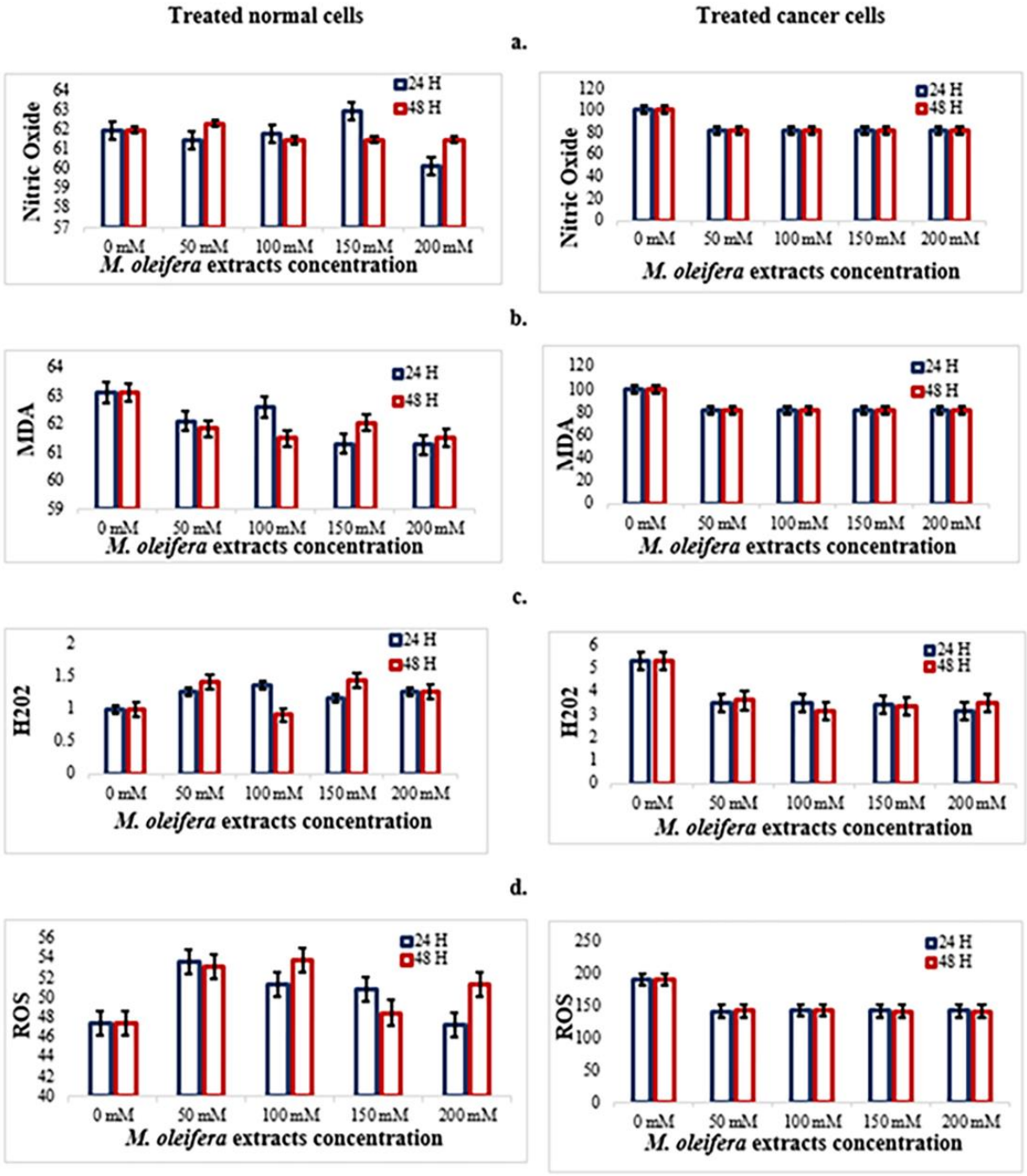

Figure 3.

Antioxidant effect of serial concentrations of *M. oleifera oil* extract on selected oxidative stress variables (a. NO; b. MDA; c. H_2O_2; d. ROS) of SH-SY5Y neuroblastoma cell line compared to treated normal cells

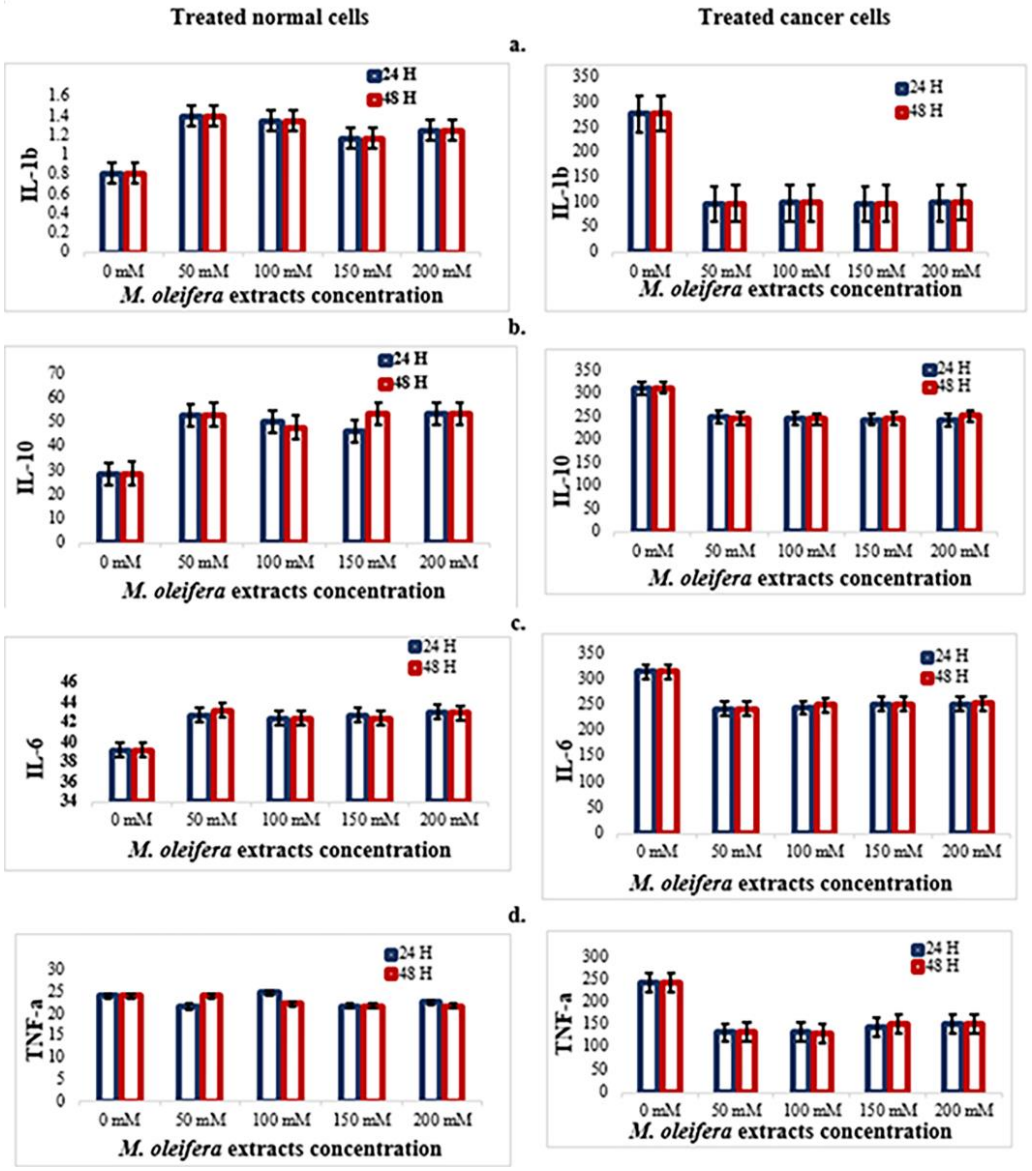

Figure 4.
Anti-inflammatory effect of serial concentrations of *M. oleifera* oil extract on selected cytokines (a. IL-1β; b. IL-6; c. IL-10; d. TNF-α) of SH-SY5Y neuroblastoma cell line compared with normal cells

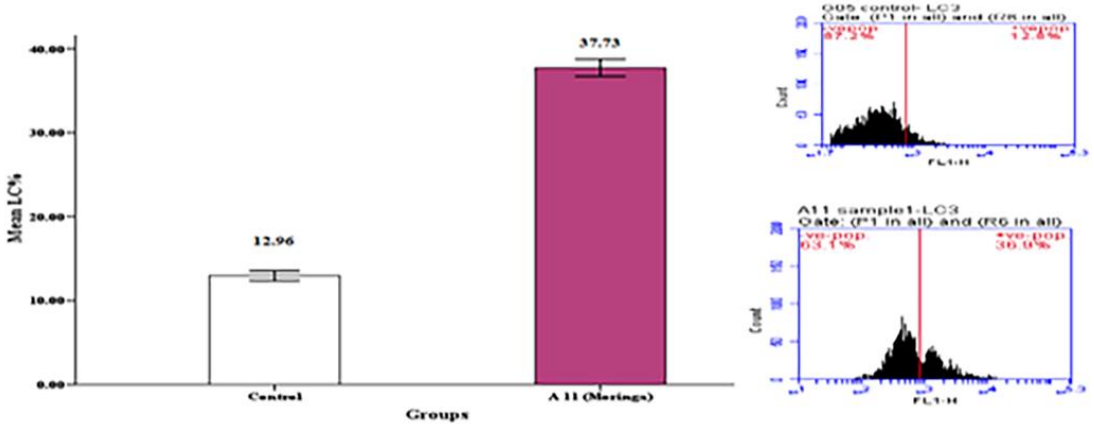

Figure 5.
Autophagic activity levels in control and *Moringa olifera* oil-treated cells

Unquestionably, moringa plant is considered one of the most important plants in complementary medicine, and it is still receiving particular attention due to its pharmacological significance as a novel nutraceutical substance for humans. The current study revealed noteworthy antioxidant and anti-inflammatory properties of *Moringa oleifera* oil against basal SH-SY5Y neuroblastoma cell line. Our findings seem to be supported by Amara *et al*. [22], who found that treating SH-SY5Y cells with Moe for 2 hrs increased the relative mRNA expression and protein quantity of NRF2 and HO.

In an obvious contradiction to the previous studies, we found that *M. olifera* had high antioxidant and anti-inflammatory effects on the SH-SY5Y neuroblastoma cell line. This may be compatible with previous study on *M. oleifera* oil's toxicity against several human cancer cells, including HeLa, HepG2, MCF-7, CACO-2 and L929 [22]. The authors showed that *Moringa oleifera* extract (Moe) prevents oxidative damage by lowering ROS formation, restoring mitochondrial respiratory chain complex activities. Moreover, Moe increased the relative mRNA expression and the protein amount of NRF2, prevented neuronal damage partly by inhibiting endoplasmic reticulum stress. The crucial difference between our work and a previous study [22], is that they recorded data based on dose and time-dependent effects of moringa extracts. In contrast, our reported opposite of that data was time and dose independent. In order to elucidate this discrepancy, it is worth noting that in their investigation, they discovered that, up to a specific dose, the extract showed toxic effect to the control sample. But over or beyond this threshold the extract showed efficacy. This phenomenon exhibited dose- and time-dependent characteristics, remaining consistent at 24, 48 and 72 hrs. This deviation from findings in several studies investigating the impact of *Moringa oleifera* on various cancers such as HeLa, HepG2, MCF-7, CACO-2, references where an exponential increase in dose-dependent pattern was observed. Central Nervous System (CNS) cancer cell lines appear to respond to drugs in a diverse manner [23]. Depending on their distinct oncogenesis modifications and tumour origins, many brain tumours may intrinsically exhibit a multidrug resistance (MDR) phenotype, eventually contributing to relapses or disease progression [23]. The interpretation of dosage and time independent could be related to resistance of NB cells, which is usually caused by comprehensive mechanisms such genetic (mutation, amplification) and epigenetic alterations (DNA hypermethylation, histone modifications), and/or several mechanisms are involved like increasing the expression of ATP-binding cassette (ABC) transporter family proteins. An effective drug must be able to pass through the cell membrane and avoid being expelled out of the cell by efflux transporters. Overexpression of efflux transporters is associated with resistance to many chemotherapeutic agents. And or aberrant expression of microRNAs (miRNAs), cancer cell stemness, autophagy, tumour microenvironment, extracellular vesicles, MEK/ERK signalling hyper-activation, and anti-disialoganglioside antibody internalisation

In other studies [11, 24]**,** the authors proposed that these antioxidant activities could be attributed to some precious unsaturated fatty acids in oils such as OA and PA. These unsaturated fatty acids have been shown to have anti-cancer properties *via* lipid peroxidation pathways and free radical production, which affects the integrity of cell membranes [24]. These results interpret the increase in MDA in the present study, which sequentially showed a prominent decrease post treatment with *M. olifera* oil 200 µg, (Figure 3). On the other hand, the current study recorded a pronounced increase in levels of TNF-α, IL-6, IL-10, IL-1β, indicating that reactive species play a role in activating astrocytes and microglia [25]. Furthermore, it can activate the associated signalling pathways, allowing the production of the previously mentioned proinflammatory cytokines [25, 26]. Remarkably, after being treated with that dose of *M. olifera* oil, these pro-inflammatory cytokines showed a noticeable decline in their measured levels (Figure 4). This implies that the fatty acid constituents of *M. olifera* oil, linoleic and/or oleic acid, may prevent inflammation by scavenging free radicals, as corroborated by some previous and updated studies [27, 28], which reported that high linoleic acid contents of *M. olifera* oil have a significant impact on oxidative stress markers and may result in a reduction in the lipid peroxidation state of the SH-SY5Y cells under basal conditions.

Although the time and dose independence as a form of resistance of neuroblastoma cell line to *M. olifera* oil, it demonstrates considerable antioxidant effects presenting as lower H_2O_2, NO, MDA and ROS. The literature has inconsistent evidence addressing the dual effects of nitric oxide in carcinogenesis and tumour growth, stressing the complexity of its involvement. Inflammation upregulates iNOS, which is connected to pro-inflammatory cytokines such as TNF-α [29, 30]. NF-κB, a prominent inflammatory pathway, plays a role in upregulating iNOS. Conversely, anti-inflammatory cytokines such as IL-10 are associated with reduced NO levels.

Recent research indicates that IL-10 predicts severity and death in acute or post-acute infection, acting as an endogenous warning signal generated by injured tissues to guard against hyperinflammation. The bimodal effects of NO and its control by multiple cytokines highlight the complex dynamics, providing both obstacles and opportunity for focused therapeutic options in cancer therapy. This was in good accord with our recorded data, which showed that in the treated NB cell line, the utilised oil dramatically

reduced NO while also inducing a notable decrease in pro-inflammatory cytokines (Figure 3 and Figure 4). Taking the fact that the brain is one of the organs that produces the highest amounts of ROS since the defensive enzymatic system in neuronal cells is poor, with minimal SOD, catalase, and GPx activity in comparison to other organs. Since glutathione, an essential antioxidant component, is found in the brain at low levels, GSH is engaged in the prevention of mitochondrial damage, cell death, and in the pathophysiology of CNS, providing evidence for the link between GSH and neurodegenerative illnesses [31]. The current study's findings may be supported by this evidence, as treatment with 200 µg modulated GSH levels. Elucidating the scavenging capability of *M. olifera* oil in relation to the complexity of GSH-mediated neuroprotective pathways. These findings may be consistent with the anti-cancer effects of exceptional fatty acid contents of moringa oil, since they can interact with cancer cell proteins and boost cancer cell growth inhibitory factors, reducing their propensity to spread to other tissues.

The effect of autophagy on cancers is dependent on multiple factors including the tumour microenvironment, the cancer type and stage, and the genetic background. The complex roles of autophagy in therapy resistance have been excessively studied. As a pro-death or pro-survival cellular process, autophagy participates in therapy resistance in various types of cancer through a bi-directional and context-dependent way [32]. The biochemical results of the present study demonstrated that *Moringa oleifera* oil activated an autophagic flux in SH-SY5Y cells, whether differentiated or undifferentiated. Our analysis shows that autophagy is present in control-untreated cells to maintain homeostasis [33] (Figure 5). These findings could imply that SH-SY5Y cells induce autophagosome formation as an early survival mechanism. The present study observed an increase or an accumulation of LC3-II, indicating the activation of autophagy. Interestingly, this activation of autophagy occurred at a physiological dose of olive oil and may have preceded apoptotic cell death. Surprisingly the rate of autophagy was positively modulated at concentration of 200 µg of *M. olifera* oil with its prestigious constituents to be in complete agreement with Jiang *et al*. [34, 35] as they reported that oleic acid as one of the moringa oil constituents induced formation of autolysosomes and decreased the expression of p26 and the ratio of LC1/LC11. These findings are compatible with prior studies by Alarcon-Gil *et al*. [27], who demonstrated that LA acts as a potent neuroprotective and anti-inflammatory agent in PD models. Moreover, they added that LA improves autophagy flux, which has an antioxidant effect in both the *in vitro* and *in vivo* PD models. These findings may direct our attention that not only moringa oil has a promising effect in neuroblastoma but also, autophagy may play a role in the basal oxidative stress in neuroblastoma cell lines, suggesting potential therapeutic strategies for treating this kind of resistant neuroblastoma.

Conclusions

Regarding the emerging emphasis on cellular autophagy, a comprehensive literature overview was conducted to discuss the pharmacological aspects of autophagy, focusing on its interplay with oxidative stress in neurological disorders. Various human diseases have been contributed to alterations in autophagy entailing cancers, the current limited and preliminary study revealed that treatment of neuroblastoma cells with physiological concentrations of *Moringa oleifera* oil rich with PA or OA and others seems to work synergistically and conferred a substantial protection against the internal oxidative insult in terms of antioxidant, ant proinflammatory. The limitations of this study should also be mentioned. Firstly, experiments were conducted on neuroblastoma, which is related to the availability of biological material, and the disease mainly affects the brain. On the other hand, fatty acids are the significant components of moringa oil, it is also worth noting that the activity the proteasome may vary with the cell line and/or organ type and the surrounding conditions; and may need to be conducted based on the treatment with the moringa oil constituents individually, therefore, it is believed that pleiotropic mechanisms and specific fatty acid interactions are involved in their beneficial effects. Furthermore, more detailed studies are needed to understand the molecular mechanisms of *Moringa* oil.

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Conflict of interest

The authors declare no conflict of interest.

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