

Review Article

Moringa oleifera: An apoptosis inducer in cancer cells

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Abstract

Moringa oleifera, usually called “wonderful tree”, has been scientifically proven to inhibit proliferation of many cancer cell types by induction of apoptosis. Apoptosis is an energy dependent process, whereby cells die for death as a result of abnormal gene mutation, ageing among others commit suicide. It is referred to as hallmark of cancer. Proliferation of cancer cells is not unconnected to deficiency in their apoptotic process such as loss of caspase activity, p53 gene mutation and imbalanced regulation of Bcl2 proteins which leads to inactivation or reduction in cancer cell apoptosis, hence, agents that are capable of inducing apoptosis in cancer cells are potential sources of effective anticancer drug. The ability of *M. oleifera* to trigger apoptosis in cancer cells largely depends on its phytochemicals, most especially antioxidant phenols such as gallic acid, chlorogenic acid, rutin, apigenin, astragaloside, quercetin, and kampferol. These compounds act by activating pro-apoptotic protein such as caspases, TRAIL, bax, bad, and inhibiting activity of anti-apoptotic proteins like Bcl2, IAPs (inhibitor of apoptosis), FLIP. In this review, we discussed cell apoptosis and its pathways, dysregulation of apoptosis in cancer cells, and more importantly, induction of apoptosis in cancer cells by *Moringa oleifera* tree.

Keywords: Apoptosis, cancer cells, *Moringa oleifera*

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INTRODUCTION

Moringa oleifera is a soft wooded tree whose fruits, roots and leaves have been advocated for medicinal and industrial uses [1]. *Moringa* preparations have been cited in scientific literatures as having hypoglycaemic, antibiotic, hypertensive, anti-inflammatory and antitrypanosomae activities [2]. It has value in tumour therapy and has been found to have the potential of preventing cancer [3]. Aqueous and solvent extracts of *M. oleifera* leaves have been reported to have significant antiproliferative effects on colon, alveolar, and pancreatic cancer cells and induce apoptosis in KB cells and other cancer cells [4-6].

Apoptosis, which is a programmed cell death, is energy required and highly regulated process

essential for normal homeostasis, and it is regarded as the hallmark of cancer [7]. Apoptotic cell is characterised by certain morphological properties at different stages of the apoptosis process. At early stage of apoptosis, microscopic view of the cell reveals chromatin condensation, nuclear fragmentation, pyknosis, rounding up of cells and pseudopods retraction [8] while late apoptosis is marked by cell membrane blebbing, cellular shrinkage, exposure of internal phosphatidylserine to the outside surface of the cell, and formation of apoptotic bodies [9].

Apoptosis is carried out via two major pathways; death receptor pathway (extrinsic pathway) and mitochondrial pathway (intrinsic pathway). Extrinsic pathway starts by binding of death receptors (e.g. Fas (CD95), TNFR1, DR4 and DR5) with their specific ligands (e.g. TRAIL and

TNF α ligands). This binding causes trimerization of the receptors and clustering of their cytosolic death domains, which in turns, permits the recruitment of adaptor molecules by the receptors. The complex formed by the attachment of ligands on the cell surface and adaptor molecules in cell cytoplasm to death receptors is referred to as Death Inducing Signalling Complex (DISC)[9-10]. DISC recruits and binds to initiator caspases (majorly caspases 8 and 10), upon binding, the caspases are

activated by cleaving due to induced proximity [11-12] (Figure 1). The activated initiator caspases execute downstream cleavage and activation of effector caspase 3, followed by apoptosis cell death.

DNA damage, oxidative stress, high concentration of cytosolic Ca²⁺ and growth factors deprivation are part of internal stimuli that trigger and initiate activation of intrinsic pathway[9]. When activated, the permeability of

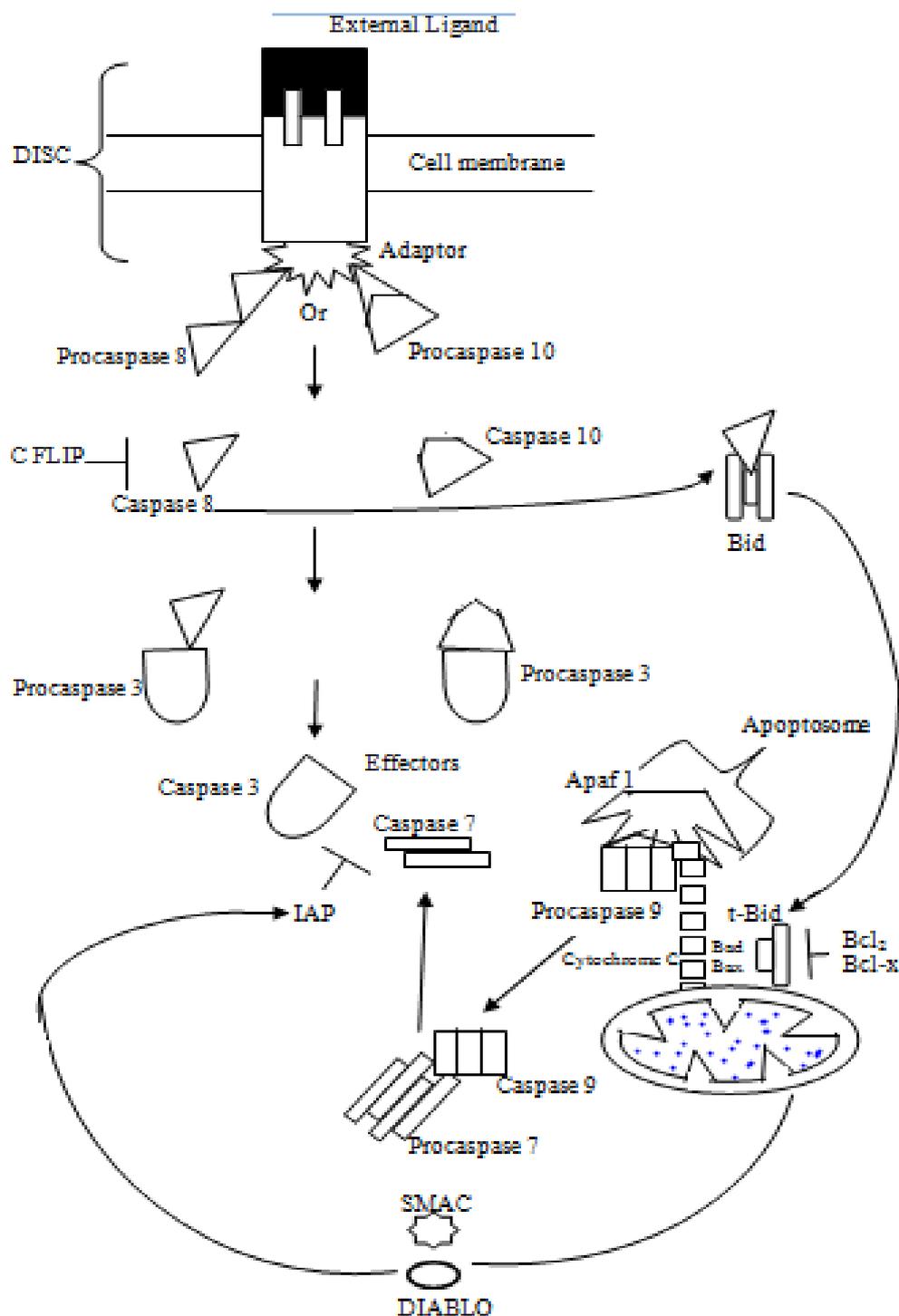


Figure 1: The major apoptotic pathways

mitochondrial membrane is compromised for the release of pro-apoptotic proteins cytochrome c, SMAC, DIABLO, among others [9, 13]. SMAC, DIABLO proteins act by interacting and binding to IAPs (inhibitors of apoptosis) in order to prevent them from binding with effector caspases (caspases 3 and 7) and inhibiting their apoptotic actions [14-15]. The release of these proteins is controlled by members of Bcl2 (B-cell lymphoma 2) protein family. Bcl2 family proteins include Bax, Bad, Bid, Bcl2, Bcl-xl, Bak etc. and are differentiated into pro-apoptotic and anti-apoptotic proteins based on their respective roles in the process of apoptosis. The pro-apoptotic proteins promote the release of cytochrome c from the intermembrane space of mitochondria to the cell cytoplasm while the anti-apoptotic proteins act by preventing such release to inhibit apoptotic death of the cell [16] (Figure 1). Summarily, the release or otherwise of cytochrome c from the mitochondria to cytoplasm depends on the resultant effect of the balance of pro-apoptotic and anti-apoptotic proteins of Bcl2 family.

When cytochrome c is released to the cytoplasm, it binds with Apaf 1 and pro-caspase 9 to form a complex, apoptosome. Upon formation of apoptosome, pro-caspase 9 is activated to caspase 9 by induced proximity at apoptosome [17-18]. Active caspase 9 cleaves caspase 7 to activate it. The activated caspase 7 then carries out apoptosis by cleaving and breaking down proteins, vital for cell survival [19] (Figure 1). Other supportive pathways that implicated in apoptosis include p53 mediated, NF- κ B, and endoplasmic reticulum pathways [20].

Tumour is brought about by dysregulation of apoptosis process which makes cell resistant to death [21]. This dysregulation of apoptosis process occurs by the following major mechanisms; loss or reduction of caspases activity [22-24], dysregulated balance of pro-apoptotic and anti-apoptotic proteins [25-26], p53 gene mutation [27], and impairment in death receptor signalling [21].

Hence, apoptotic inducers (such as *M. oleifera* phytochemicals) in cancer cells are potential effective chemotherapeutic agents for cancer. The rest of this article discuss the description *M. oleifera* plant, chemical compounds of the plant that are capable of inducing apoptosis in cancer cells, and more importantly, reported research works that proved *M. oleifera* to be apoptosis inducer in cancer cells.

***Moringa oleifera* is a “wonderful tree”**

M. oleifera plant, also known as horseradish tree and drumstick [28-29], is fondly called “wonderful tree” or “miracle tree” because of its numerous medicinal, nutritional and economic values [30]. It is believed that the plant originated from Himalayan foothills, India [31-32]. It belongs to a family of 14 species called Moringaceae [28] and most widely studied and cultivated species all over the world as it can now be found in many countries like Pakistan, Thailand, Malaysia, Indonesia, North American countries and West African countries among others [28, 31].

M. oleifera is a deciduous plant [28] that has flexible minimal growth condition requirements as it can thrive well in environments with wide range of rainfall abundance and hot dry lands [33]. It has a height of 5 to 10m [30, 33] and its bipinnate or tripinnate leaves are usually evergreen while its long pods enclose the plant seeds [28]. The seed is encapsulated by hull and it appears green at immature stage. When matured, the pods become dry and brown and the solid seed turns white with sweet taste [28, 34].

The leaves and seeds of *M. oleifera* are edible and in fact, form part of normal diets in some countries; they can be eaten raw, cooked or added to curries [35]. The leaf is said to be extraordinarily nutritious as it has more vitamin C than orange, high protein quality as that of eggs and milk, more potassium than banana, higher vitamin A content than carrot, and high calcium content than milk [3]. The plant is a source of natural coagulant with significant water purification power. When added in raw or semi-refined form to turbid and dirty water, it clears and purifies it by settling dirt and other debris at the bottom of the water [36]. Meritoriously, in vivo and in vitro studies showed that the water purified by *M. oleifera* seeds (<2mg/ml) has no or insignificant toxicological effect and such water are safe for drinking [37].

M. oleifera has many medicinal values as research had shown that it possesses antimicrobial, anti-inflammatory, hepatoprotective, antiulcer, antidiuretic activities [38-41] and more importantly to this review, preclinical studies proved it has growth inhibitory effect to proliferation of many cancer types [4-6]. For example, Charoensin [42] carried out a two-stage successive extraction of phytochemicals of *M. oleifera* leaf using methanol, and dichloromethane solvents. The two extracts were tested on breast cancer cells (mcf7), colorectal cancer cells (Caco-2), and hepatocellular carcinoma

(HepG2) to determine their growth inhibitory efficiency. The extracts significantly inhibit proliferation of the cancer cells in dose dependent manner and cell viability assay of normal human dermal fibroblast treated with the extracts showed the cell growth is unaffected.

Ability of *M. oleifera* to Induce Apoptosis in Cancer Cells

The potency of *M. oleifera* to induce apoptosis in cancer cells largely depend on antioxidant capacities of its phytochemical constituents which are majorly natural phenolic compounds [43-45]. These antioxidants (phenols) are chemicals capable of scavenging free radicals or stabilize free reactive oxygen species to prevent oxidative stress within the cell environment [46]. Oxidative stress is responsible for many degenerative diseases such as Alzheimer, Parkinson, Huntington, and cancer [47].

Abundant research based works have proven different antioxidants to be apoptosis inducers in cancer cells [48]. Antioxidants, especially phenols influence apoptotic signalling pathways to bring about cell death by activating pro-apoptotic proteins and inhibiting anti-apoptotic proteins action [44]. For instance, curcumin (antioxidant natural phenol) induces apoptosis in many cancer cells by activating caspase 3 and 7 and inhibiting Bcl2 from preventing the release of cytochrome C from mitochondria [44, 49]. Berberine, another antioxidant, triggered fas activity to promote apoptosis in HepG2 cells [50]. *M. oleifera* has enormous amount and varieties of phenols. Three primary phenolic compounds, isoquercetin, crypto-chlorogenic acid and astragalgin were detected in leaves of *M. oleifera* by High Performance Liquid Chromatography (HPLC) [51]. More recently, HPLC-DAD-ESI MS/MS analysis carried out by Karthivashan et al. [52] confirmed the presence of multiflorin-B, apigenin-8-C-glucoside, quercetin and kampferol derivatives in crude hydro-ethanolic extract of *M. oleifera* leaves. Similarly, 7 polyphenols namely; gallic acid, chlorogenic acid, luteolin, rutin, quercetin, kampferol and apigenin were identified in methanolic extract of *M. oleifera* by HPLC analysis [53]. Also, reproductive and vegetative tissues of *M. oleifera* leaves and stem were found to have quinic acid, quercetin and kampferol [54].

Moreover, DPPH, FRAP, ABTS and other assays had been used to determine antioxidant capacities of various part of *M. oleifera* plants. The results revealed it has high and significant antioxidant capacities. A comparative analysis between DPPH free radical scavenging activities

of *M. oleifera* and some selected vegetables (spinach, cauliflower, cabbage and peas) revealed leaves and flower of the plant have higher free radical scavenging activities than all the vegetables. In the same report, total phenolic and flavonoid contents of *M. oleifera* leaves and flower are at least two times higher than that of the vegetables suggesting that the antioxidant capacities of the plant's part may be due to their phenolic compound contents (phenolic acid and flavonoid) [55]. Similarly, Sreelatha and Padma [56] reported that mature and tender leaves of *M. oleifera* have significant antioxidant activities with IC₅₀ 18.15 ± 0.92 µg/ml and 19.12 ± 0.75 µg/ml respectively which correlated with their total phenolic and flavonoid contents.

In addition, radical scavenging ability of leaf and seed tissues of *M. oleifera* was evaluated and it was confirmed that ethanolic extract of the plant's parts have better scavenging capacity than saline extracts. Furthermore, a comprehensive work on total phenolic content and antioxidant capacities of leaves, pods and seeds of *M. oleifera* using Folin-Ciocalteu assay for phenolic content quantification and FRAP, DPPH, and ABTS assays to determine antioxidant activities revealed that all the plant's parts have great amount of phenolic acid and significant antioxidant capacities with leaves having highest amount [57].

Induction of apoptosis by *M. oleifera* in cancer cells

Though, there is no clinical trial report on anticancer efficacy of crude extracts or isolated compounds from *M. oleifera*, however, several preclinical research works had confirmed the ability of seed, leaf and bark of *M. oleifera* to induce apoptosis in many cancer cells leading to their ultimate death by controlling activation/inhibition of apoptosis biomarkers of clinical importance (Table 1). Cold soluble aqueous extract of *M. oleifera* leaves had been shown to trigger apoptosis in A549 lung cancer cells via mitochondrial mediated pathway by activation of pro-caspase 3 to caspase 3. Additionally, in the same study, it was observed that A549 cell membrane integrity was compromised as propidium iodide (PI) was able to pass through it and got attached to nucleic acid in the cytoplasm [6]. In a related observation, Tiloke et al. proved hot water extract of *M. oleifera* leaves to be apoptosis inducer in A549 cancerous alveolar epithelial cells by upregulating expression of pro-apoptotic proteins, p53, Smac/DIABLO, downregulating Nrf2 anti-apoptotic protein expression, and cleavage of PARP-1, furthermore, increased activities of caspases 3,7

Table1: Induction of apoptosis by different parts of *M. oleifera* in various cancer cells

Plant part	Extraction solvent	Targeted biomarker	Cancer type	References
Leaf	95% ethanol	Apoptotic bodies	HeLa cancer cells	Hermawan, <i>et al.</i> , 2012 [61]
	Cold water	Characteristic DNA fragments due to apoptosis, Cleaved PARP, caspase 3, and Bcl-xL	HepG2 liver cancer cells	Jung <i>et al.</i> , 2015 [60]
	Ethanol	Exposed phosphatidylserine at the outer surface of cell membrane due to compromise of cell membrane integrity	MDA-MDB 231 breast cancer and HCT-8 colorectal cancer cells	Al-Asmari <i>et al.</i> , 2015 [59]
	Hot water	Characteristic DNA fragment, cells permeable to propidium iodide	KB tumour cells line of glandular cancer of cervix	Sreelatha <i>et al.</i> , 2011 [4]
	Water	p53, Smac/DIABLO pro-apoptotic proteins and Nrf2 antiapoptotic protein	A549 lung cancer cells	Tilokeet <i>et al.</i> , 2013 [58]
	Cold water	Caspase 3	A549 lung cancer cells	Jung, 2014 [6]
	Hot water	p65, 1k β α and phosphor-1k β α	Panc-1 pancreatic cancer cell	Berkovichet <i>et al.</i> , 2013 [5]
Seed	Ethanol	Exposed phosphatidylserine at the outer surface of cell membrane due to compromise of cell membrane integrity	HCT-8 colorectal cancer cells	Al-Asmari <i>et al.</i> , 2015 [59]
Bark	Ethanol	Exposed phosphatidylserine at the outer surface of cell membrane due to compromise of cell membrane integrity	MDA-MDB 231 breast cancer and HCT-8 colorectal cancer cells	Al-Asmari <i>et al.</i> , 2015 [59]

and 9 were observed in treated A549 cells [58].

In addition, phytochemicals extracted by hot water (80°C) from *M. oleifera* leaves triggered apoptosis in KB tumour cells. PI and DAPI staining of the treated cells revealed that the

cells were permeable to PI which suggests that apoptosis had occurred, moreover, DAPI staining confirmed the presence of characteristic nuclear changes peculiar to apoptotic cell death such as the appearance of fragmented and condensed chromatin [4].

Also, hot water extraction of leaves of *M. oleifera* induced apoptosis in Panc-1 pancreatic cells by downregulating expression of essential proteins of NF- κ B signalling pathway after 24 hours treatment. p65, 1 κ B α and phosphor-1 κ B α were downregulated in treated cells as compared with untreated control cells [5].

Using annexin V and PI dual staining techniques whereby annexin V binds to translocated phosphatidylserine (PS) on the outer surface of cell membrane caused by apoptosis and PI passes through cell membrane when the membrane integrity has been compromised due to necrosis cell death and intercalate into nucleic acid of cell cytoplasm, Al-Asmari *et al.* showed that ethanolic extracts of *M. oleifera* leaves and bark induced apoptosis in MDA-MDB-231 breast cancer cells and HCT8 colorectal cancer cells while ethanolic extract of the plant seeds mildly triggered apoptosis in HCT8 cells only [59].

In addition, a potential oral anticancer drug of cold water extract of *M. oleifera* leaves induced apoptosis HepG2 human hepatocellular carcinoma cells by activating caspase 3 via cleavage and downregulating the expression of Bcl-xL anti-apoptotic protein [60]. In addition, Hermawan *et al.* detected apoptotic bodies formed in HeLa cells when treated with ethanolic extract of leaves of *M. oleifera*, the apoptosis cell death was further enhanced in the cells when treated with a combined drug of 100nM doxorubicin and 250 μ g/ml of the leaf extract [61].

CONCLUSION

Undoubtedly, abundant research works on apoptosis in different cancer cells clearly suggest that impairments along apoptotic pathways are major causes of proliferation and metastasization of tumour cells. So, treatment strategies targeting the restoration of normalcy of apoptosis in cancer cells may be the adequate remedy to cure cancer. *Moringa oleifera*, as it has been proven in the above cited literatures, is a good and efficient source to further explore in order to find potent compounds that will be harmless to normal body cells and effectively kill or restore cancer cells to normal. Preliminary works that confirmed anticancer activities of different parts of the plant like leave, seed, bark and root are numerous; however, further molecular analyses are required to understand the mechanisms of action of the plant parts. This is important to achieve the terminal goal of discovering efficient and harmless therapy for cancer.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. IAA and HA conceived and designed the study while all authors prepared and approved the manuscript for publication.

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