MOLECULAR TARGETS OF *MORINGA OLEIFERA* IN CANCERS (*IN VITRO* AND *IN VIVO*): A SYSTEMATIC REVIEW

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Abstract

Cancer is unquestionably the greatest worldwide threat to humanity. With fewer side effects, natural anticancer medicines are the most prevalent. Anticancer benefits of dietary polyphenols could be achieved through carcinogen elimination, cancer cell signalling alteration, antioxidant enzymatic activities, and apoptosis-inducing substances, according to the likely approach to anticancer effects of dietary polyphenols research. This study aims to investigate the apoptosis induction mediated by Moringa oleifera in in vitro and other molecular targets *in vivo* in all types of cancer studies, as well as to describe the most recent and comprehensive research on antioxidant and anticancer characteristics. The terms "molecular target", "signalling pathway", "apoptosis", "*Moringa oleifera* extract", "mechanism of action", "cancer", "tumour", "carcinoma", and "melanoma" were used to search four online databases, PubMed, Scopus, ScienceDirect, and Google Scholar, for relevant articles published between 2009 and 2019. There are a total of forty-four concluding pieces in this review. It was revealed that, among all plant extracts, had the highest cytotoxicity against cancer cells and the lowest cytotoxicity against normal cells. Anticancer processes, such as the intrinsic apoptosis pathway and the regulatory expression of the Bcl- xvi 2 family, which contains anti-apoptotic and pro-apoptotic proteins, have been revealed by more research. Phenolic compounds derived from crude extracts of Moringa oleifera, such as quercetin-3-O-glucoside, prevent DNA damage by decreasing reactive oxygen species. This resulted in less cellular mutation and cancer being produced. With fewer adverse effects, natural anticancer agents predominate. Polyphenols are important and widely utilized; the likely approach to anticancer effects of dietary polyphenols research stated that they would be accomplished through carcinogenic elimination, cancer cell signalling modification, antioxidant enzymatic activities, and apoptosis induction agents. This review seeks to explore the signalling pathways mediated by Moringa *oleifera* in all *in vivo* and *in vitro* cancer investigations, as well as summarize the most recent and thorough research on antioxidant and anticancer properties. Using the search terms "molecular target", "signalling pathway", "Moringa oleifera extract", "mechanism of action", "cancer", "tumour", "carcinoma", and "melanoma", four online databases, PubMed, Scopus, ScienceDirect, and Google Scholar, were systematically searched for relevant articles published between 2009 and 2019. This review has a total of forty-four concluding articles. It was discovered that, among all plant extracts, exhibited the highest cytotoxicity against cancer cells and the lowest cytotoxicity against non-cancerous cells. Additional research has elucidated the potential anticancer processes, such as the intrinsic apoptosis pathway and the regulatory expression of the Bcl- xvi 2 family, which includes anti-apoptotic and pro-apoptotic proteins. As a result of preventing DNA damage, phenolic compounds from crude extracts of Moringa oleifera, such as quercetin-3-O-glucoside, contribute to the decrease of reactive oxygen species. Consequently, it decreased the rate of mutation and the development of cancer cells.

Keywords: Carcinoma, Mechanism of action, Melanoma, Molecular target, *Moringa oleifera* extract

Introduction

Cancer appears to be a global killer that claims more lives than any other disease, despite the remarkable advances in scientific and treatment technologies (1). *Moringa oleifera* is a well-known medicinal herb, particularly in underdeveloped nations. It has been utilized as a food and medicinal plant in Asia, particularly in India and Southeast Asian nations (2). *Moringa oleifera* has been dubbed a "wonder tree" since all its parts (leaves, skin, buds, roots, and seeds) can be used to prevent and treat a variety of ailments. Therefore, it has always been used as an anticancer, anti-inflammatory, antihypertensive, antibacterial, anti-diabetic, and antioxidant agent, and it also has hepatoprotective and cardioprotective properties (3). *Moringa oleifera* also possesses significant therapeutic benefits throughout its entirety, particularly in its leaves, which are rich in antioxidant vitamins C and E, minerals such as potassium, iron, and calcium, and proteins (like methionine, cystine, lysine, and tryptophan). In addition, the leaves contain phytochemicals like as flavonoids, alkaloids, and carotenoids that play an essential role in disease regulation. *Moringa oleifera* contains benzyl glucosinolate 4-(-L-rhamnopyranosyloxy), benzyl isothiocyanate, and benzyl oleate (niazimicin containing Thiocarbamates). Studies suggested that niazimicin was responsible for the chemopreventive and anticancer properties of *Moringa oleifera*. The leaves also include kaempferol-3-O-glucoside and quercetin-3-O-glucoside, which are crucial in the battle against free radicals; thus, they alleviate oxidative stress and serve a crucial function as an antioxidant's agent. Moringa oleifera is one of the possible therapeutic plants with anticancer properties. Priority should be given to identifying the molecular target pathway and reason for using *Moringa oleifera* in the prevention and treatment of cancer. A systematic assessment of the molecular targets of *Moringa oleifera* in malignancies would be beneficial to society, given the importance of the traditional medicinal herb in cancer prevention and therapy. This systematic study aimed to provide an overview of *Moringa oleifera* and investigate the molecular pathways associated with cancer in this plant.

Materials and Methods

Search strategy

Based on the research title molecular targets of *Moringa oleifera* in cancers, a list of key terms was constructed for this systematic review's article identification. Four internet databases were utilized, including Google Scholar, Science Direct, PubMed, and Scopus. "molecular target," "signaling route," "*Moringa oleifera* extract," "mechanism of action," "cancer," "apoptosis," "tumor," "carcinoma," and "melanoma" were the search terms used.

Inclusion and exclusion

The inclusion criteria for selecting relevant studies were as follows: molecular experiments on the effect of (whole part) on cancer, articles published between 2009 and 2019 (10 years period). *Moringa oleifera* as one of the roles for their anticancer effect, study designs using *in vitro*, *in vivo* and *ex vivo*. Articles which were written in language other than English, and articles that reported the activity of *Moringa oleifera* from unknown source were excluded.

Data extraction

The predefined keywords were used to search web databases for articles, which were then screened, and duplicates were deleted. The author did the screening based on inclusion/exclusion criteria (document type, timeline, and language). For further data, a manual search of additional sources, such as the reference list 18 of papers and forward citations, was done. The eligibility was determined by hand by the author.

Results

According to *in vitro* and *in vivo* evaluations, *Moringa oleifera* is one of the most promising medicinal plants for preventing and treating cancer, as suggested by all the literatures reviewed in this study. One of the most widely used natural compounds against cancer is polyphenols found in plants. The most important anticancer mechanism of dietary polyphenols is the induction of apoptosis. However, the reviewed literature has several shortcomings. Regulating the threshold value of *Moringa oleifera* active compounds will require additional research. Estimation of the quantities of these polyphenols required for efficient and beneficial impact against multiple forms of cancer. To demonstrate the toxicity of *Moringa oleifera*, extensive research is also required. This systematic review aims to categorize, evaluate, and summarize the findings of all relevant studies on a health-related topic, making the prevailing evidence more accessible to decision-makers such as physicians, pharmacists, and researchers. In conclusion, we anticipate that this systematic review will serve as a reference and fill the knowledge gap for researchers to design *in vivo* and clinical studies on *Moringa oleifera* to raise awareness about the development of novel pharmaceutical agents for preventing and treating cancer.

Discussion

Literature search

The literature searched conceded a total of 190 records (from the year 2009 to the year 2019). The numbers of articles obtained from the corresponding databases are as follows; Scopus (n=105), PubMed (n=23), Science Direct (n=30), Google Scholar (n=32). A total of 30 duplicate articles were removed and 160 articles remained. Seventy-three articles were excluded based

on title and abstract criteria. Eighty-seven full-text articles were assessed for eligibility; 35 of them were excluded from review. Thus, the final number articles that met the inclusion criteria was 44.

Distribution of selected articles according to country and year of publication

There is a substantial correlation between the number of studies completed on and the geographical distribution of *Moringa oleifera*. Most research on *Moringa oleifera* (7 papers) was undertaken in Malaysia, followed by India (6 articles). From Italy, South Korea, South Africa, and Saudi Arabia (3 articles). Thailand and Indochina (2 articles). Balanced are from different nations, including Egypt, France, and the United States (USA). The focus of this study is on articles published between 2009 and 2019 (10 years period).

In the years between 2009 and 2014, researchers had an initial interest in the study of *Moringa oleifera*. The pinnacle of this plant's research occurred between 2015 and 2019.

Classification of selected articles according to the study models and part of Moringa oleifera

There were thirty-seven articles pertaining to *in vitro*, three articles of *in vivo*, and four articles of both designs. From forty-four articles, nineteen studies used leaves of *Moringa oleifera* in the experiments, and twelve studies used seeds of *Moringa oleifera*, while other studies focused on the other different parts of plant. Part of *Moringa oleifera* used in this study is depicted in Figure 1.

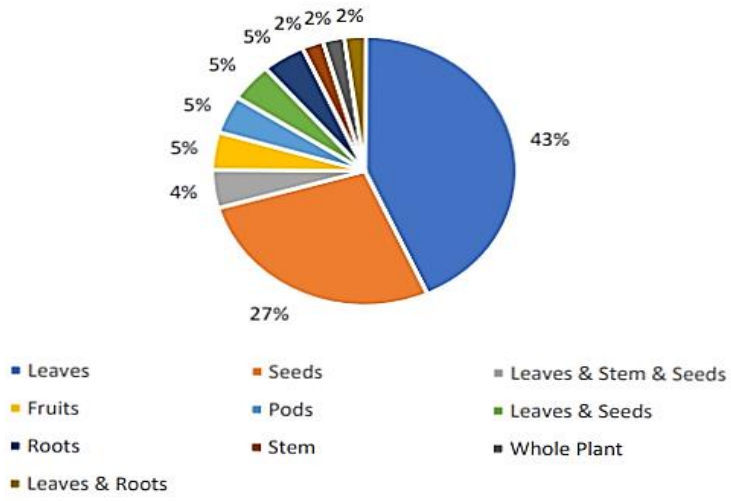

Figure 1: Part of *Moringa oleifera* used in this study

Types of Moringa oleifera extraction

The researchers preferred water as the extracting solvent because it's considered as greenest solvents, non-harmful to health, low cost for production purpose and decrease the environmental toxicity (4). A recent study demonstrated that phytochemical components and antioxidant activity of *Moringa oleifera* Lam leaves were affected by the age of the plant and extraction solvents. They used fresh leaves aged thirty, forty-five, and sixty days and three solvents: water, methanol, and ethanol.

The result showed that the methanolic and ethanolic extracts of forty-five days old showed higher antioxidant activity compared to water extracts (5). In this research most researchers had used water extraction; the average rate was 39 per cent. In comparison, methanol yields 18%, and n-Hexane only yields 9% when used as an extractant. Figure 2 shows the various methods used to extract Moringa oleifera as a percentage.

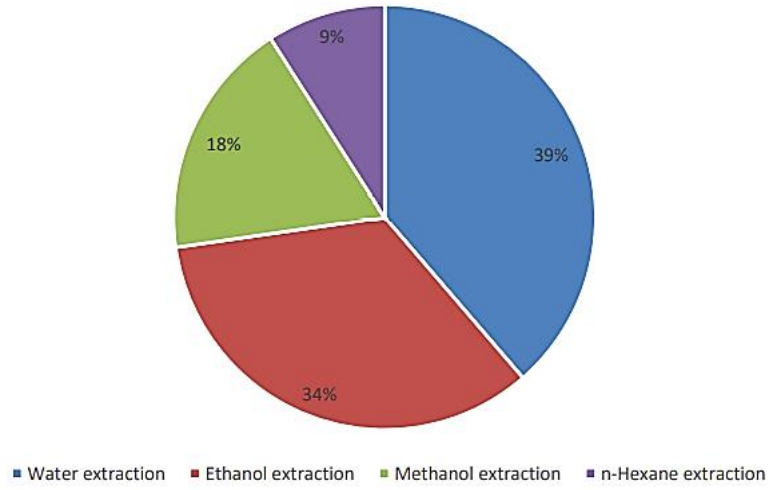

Figure 2: Types of Moringa oleifera extraction used

Induction of apoptosis by Moringa oleifera through in vitro studies

Induction of apoptosis on human hepatocellular carcinoma (HepG2 cells)

Moringa oleifera leaf (MOL) extracts have been tested in human hepatocellular carcinoma HepG2 cells. Cell cycle results depicted that MOL induced sub-G1 fraction of up to 96 per cent compared to the control group. *Moringa oleifera* water leaves extracts with dose-dependent manner displayed DNA strand breaks that were identified by TUNEL-positive cells with fragmented DNA in their nuclei by a green fluorescence signal using TUNEL staining assay to detect apoptotic morphology in human hepatocellular carcinoma HepG2 cells (6). A recent study by Tiloke et al. (2019), demonstrated the ability of the crude aqueous extract from *Moringa oleifera* leaves in initiating apoptosis and cell-cycle arrest in cancerous HepG2 cells, through augmented DNA damage, lipid peroxidation also γH2AX levels, moreover, reduce in G2-M phase, G1, and S, in addition to the increase in SRp30a protein expression triggered caspase-9. The decrease in ATP level leads to increase Caspase-9 and -3/7. finally, reduction in c-myc, p-Bcl2 and Hsp70 protein and rise in Bax, Smac/DIABLO and PARP-1 cleavage which further confirmed apoptosis process (7).

Induction of apoptosis on human colon cancer cell line (HCT 116)

MAPK (mitogen-activated protein kinases) proteins responsible for the primary cellular roles are serine-threonine kinases including p38, NH2-terminal kinase, c-Jun, and ERK (extracellular signal-regulated kinase). Mutations in human cancer highly regulate the RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways. ERK signaling pathways have been shown to regulate various proteins that accelerate the development of cancer cells and growth of tumor's. *Moringa oleifera* leaves in their research have shown potent anti-proliferative properties by decreasing the ERK1/2 phosphorylation in HCT116 colon cancer cells. The findings indicated that *Moringa oleifera* leaf extract suppressed the cell growth in HCT 116 cells and displayed promising used as an anti-cancer agent (8). Guon and Chung et al. (2017), in their study utilized the extracts of *Moringa oleifera* dried fruits and their flavonoid (compound 1 rutin) (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1-6)-β-D glucopyranosyloxy] -4Hchromen-4-one and (compound 2 quercetin) (2-(3,4-dihydroxyphenyl)-3,5,7- trihydroxy-4Hchromen-4-one). Compounds 1 and 2 have shown the ability to suppress the propagation of HCT116 colon cancer cells in a dose-dependent manner. Apoptosis is considered by a sequence of morphologic changes and chromatin concentration of the cell nucleus by special mechanisms. The study also showed that treatment with MO and its compound displayed a small shrinkage of the cells and a reduction of cell number. In addition, after HCT116 cancer cells treated with *Moringa oleifera* dried fruits extracts and quercetin, Bcl-2 expression reduction was observed, followed by caspase-9 and caspase-3 activation, and PAPR (poly ADP-ribose polymerase) was identified. This signal induced apoptosis through the mitochondrial pathway. Apart from that the findings also showed that *Moringa oleifera* fruits and their flavonoids exhibit the reduction of the viability of human colon cancer cells HCT116 by 150 µg/mL in concentration-dependent manner. Thus, suggesting that this plant might be significantly toxic to the cells (9).

Reda et al. (2017), demonstrated that *Moringa oleifera* leaves water extracts have shown to reduce the viability of colon cells (HCT116, CACO2, and HCT116P53- /-) in a concentration-dependent manner varying from 0.1 to 2.5 per cent, via allocation of membrane integrity and cell cycle arrest in subG0 phase. Increased in oxidative stress and stimulate ROS producing and lead to LDH (is a cytosolic enzyme led to oxidizes l-lactate to pyruvate) emission. The release from the cytoplasm to the medium, therefore, displayed changes in the

permeability of the plasma membrane elicit the incidence of necrosis or apoptosis (10).

Induction of apoptosis on human pancreatic cancer cell line (PANC-1)

Dose-dependent *Moringa oleifera* leaves extracts with PANC-1 cancer cell revealed a reduction of Bcl-2 pro-apoptotic protein, a down-regulation of the key component of PARP-1, COX-2 (apoptosis-related proteins), p65-subunit and NF-κBrelated proteins IκB-α, respectively. The findings indicated that *Moringa oleifera* leaf extract alone and combination with radiation was showed to inhibit this cell cancer via dose-dependent manner (11). A study by Berkovich et al. (2013) prove that *Moringa oleifera* leaves extracts inhibited the growth of all pancreatic cell lines namely Panc-1, p34, and COLO 357 by decreasing the expression of IkBα, p65 and p-IkBα proteins (all three proteins of the NF-κB signaling pathway) (12). Similar findings with Hagoel et al*.,* 2019 was found where this extract also induced a boost in the sub-G1 in a dose-dependent manner. In addition, *Moringa oleifera* leaf extract via inhibition of the NF-κB pathway may increase the efficacy of cisplatin resulted in strong synergistic effects. These findings indicated that ≥ 0.75 mg/ml of the *Moringa oleifera* leaf extract result in the prevention of the development of cancer cells and raise the effectiveness of chemotherapy in human pancreatic cancer cells (11).

Induction of apoptosis on murine melanoma cell line (B16F10)

Moringa oleifera Lam all parts aqueous extracts lead to a significant decrease in development and growth rate in murine B16F10 melanoma cells, cell cycle arrest, increase of p53, p21WAF1/Cip1 and p27Kip1 protein levels and differential in induction. This essential finding led to the antiproliferative and the antioxidant event which significantly inhibit tumour growth (13). De Andrade Luz et al. (2017) in their study demonstrated the ability of Lectin (cMoL) (carbohydrate-binding proteins) extract from *Moringa oleifera* seeds in the cytotoxicity event in B16F10 murine melanoma cancer cells via promoting caspases 9, 8 and 3 activations (lectin induces caspase-dependent apoptotic cell death) and mitochondrial ROS (reactive oxygen species) production. Lectin has been shown to be less cytotoxic to GN cells (14).

Induction of apoptosis on human lung adenocarcinoma cell line (A549)

Moringa oleifera leaves extracts exhibit a decreasing effect in Nrf2 protein expression and a significant increase in ROS correlated with a drop in intracellular glutathione levels in tumorous A549 lung cells. It also has the ability to significantly increase the pro-apoptotic p53 protein expression, p53 mRNA expression, caspase-3/7 and caspase-9. The extract also enhanced the expression of Smac/DIABLO and activation of PARP-1. Therefore, *Moringa oleifera* leaves extracts was showed to potently inhibit the antiproliferative effect on cell cancer (15).

Induction of apoptosis on Human Brain Astrocytoma cells (CCF-STTG1)

The glycosylated isothiocyanate moringin [4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate] isolated from seeds of this plant has produced significant results in cell death in grade IV human astrocytoma CCF-STTG1 cells often known as (GBM) glioblastoma multiforme, and induction of apoptosis. Through Bax & p53 stimulation, and Bcl-2 suppression. A significant reduction in 5S rRNA was observed after moringin treatment. Moringin demonstrated oxidative stress reduction associated by Nrf2 transcription factor and its upstream controller CK2 alpha expressions were observed. The findings indicated that Moringin extract from *Moringa oleifera* Lam seeds has been shown to be effectively inhibit apoptosis in IV CCF-STTG1 cells of human astrocytoma (16).

Induction of apoptosis on Human Leukemia Monocytic Cell Line (THP1)

Moringa oleifera stem bark extract has been shown to potently inhibited the expression of pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β in lipopolysaccharide (LPS) stimulated by THP-1 cells. Significant suppression of the generation of nitric oxide (NO) and ROS (reactive oxygen species) prove that it can be considered as a moderator of the immune response in a dose-dependent manner (17). A recent study by POTESTÀ et al. (2019) showed the pro-apoptotic effect of *Moringa oleifera* seeds aqueous extracts in Human THP1 monocytoid and Jurkat E6- 1 lymphoid cell lines. This finding supported by the downregulation of sirtuin-1 (SIRT1). This may potentially link to microRNA existing in the extracts. The findings indicated that various aqueous extracts from leaves and seeds of *Moringa oleifera* Lam portrayed the pro-apoptotic and cytotoxic activities in Human Jurkat E6-1 lymphoid and THP1 monocytoid cell (18).

Induction of apoptosis on human colon adenocarcinoma cell line (HT-29)

A study by Ezhilarasi et al., 2016 displayed that *Moringa oleifera* leaves extracts prove has potent cytotoxic activity versus HT-29 cancer. This finding was supported by reduction of ROS (reactive oxygen species) thus led to the destruction of the cell protein, cell membrane, and DNA which then cause cell demise. The used of this plant was parallel to eco-friendly and cost-effective advantage. They also claimed that NiO nanoparticles is effective in causing toxic to the cytotoxic HT-29 colon cancer cell lines (19).

Induction of apoptosis on a panel of cell lines

Omoringone, a very recent isolated component extracted from *Moringa oleifera* leaves has shown to possess an inhibitory effect on CYP3A4, one of major drug-metabolizing enzymes of Cytochrome P450 with no significant effect on CYP2D6 isozyme. The secondary metabolites from the plant were assessed for their cytotoxicity against a panel of cell lines (SK-OV-3, VERO, LLC-PK1, and HepG2) resulting in suppressing the effects in cancer cell lines and on CYP3A4 isozyme (3). A study by Abd-Rabou et al. (2017), showed that nanocomposites of *Moringa oleifera* leaves and roots core and outer parts extracts inhibit the proliferation of different cancer cells. List of cancer cells that have been used includes MCF7, HCT 116/ Caco-2, and HepG2 cells with different ratios. Result showed that the root inhibited 70-80 per cent the plurality of cancer cells, and 30-40 per cent for normal BHK-21 cells. Apoptotic cell increment result confirmed the cytotoxic effects of these extracts (20). Elsayed, Sharaf-Eldin and Wadaan et al. (2015), demonstrated that *Moringa oleifera* seed's essential oil plays an important role in cytotoxic activities against HepG2, Caco-2, MCF-7, HeLa, and L929, mouse fibroblast cell lines. The decrease depended on the oil concentration applied as well as the cell line. Thus, the most affected cells were HeLa cells with cell toxicity verified 76.1 per cent, HepG2 65.1, MCF-7 59.5 per cent, L929 57.0 per cent, and CACO-2 49.7 per cent. This study was delivers initial step for more studies of the possible mechanism of *Moringa oleifera* seed oil on cancer cell lines (21). A similar study approved that phenolic compound namely quercetin-3-O-glucoside, sitosterol, lutein and 4-(β-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)-benzyl isothiocyanate display potent cytotoxicity in Caco-2 and HepG2 cells with moderate cytotoxicity on normal cell line which is Human Embryonic Kidney (HEK293).

Result depicted that quercetin-3-O-glucoside worked on the elimination of ROS preventing DNA damage and lead to the decreasing of mutation rates and incidence of tumour cells. In contrary, 4-(β-D-glucopyranosyl-1→4-α-L rhamnopyranosyloxy)-benzyl isothiocyanate has shown to down-regulate Bcl-2 and up-regulate Bax (22). This plant and its compounds exert its inhibitory effect on apoptosis by targeting several apoptosis proteins. Excitingly, we have found that different isolated compounds enabled targeting different types of apoptosis proteins in different types of cancer cell lines.

Induction of apoptosis on in monocyte/macrophage-like cells (RAW264.7)

The ethyl acetate fraction of *Moringa oleifera* leaves evinced to have the suppressor effect on lipopolysaccharide (LPS) stimulate production of proinflammatory cytokines and nitric oxide (one of important mediator associated with acute & chronic inflammatory diseases) by concentration-dependent manner in macrophages via suppression NF-κB signaling pathway (by averting IκBα degradation and translocation of the NF-κB p65 protein into the nucleus). This fraction has a potent effect in downregulating the expression of inflammatory mediators including inducible nitric oxide synthase (iNOS), (COX)-2 (23). Flow chart in Figure 3 depicted the ethyl acetate fraction roles in the inhibition of inflammatory in RAW2647 cells.

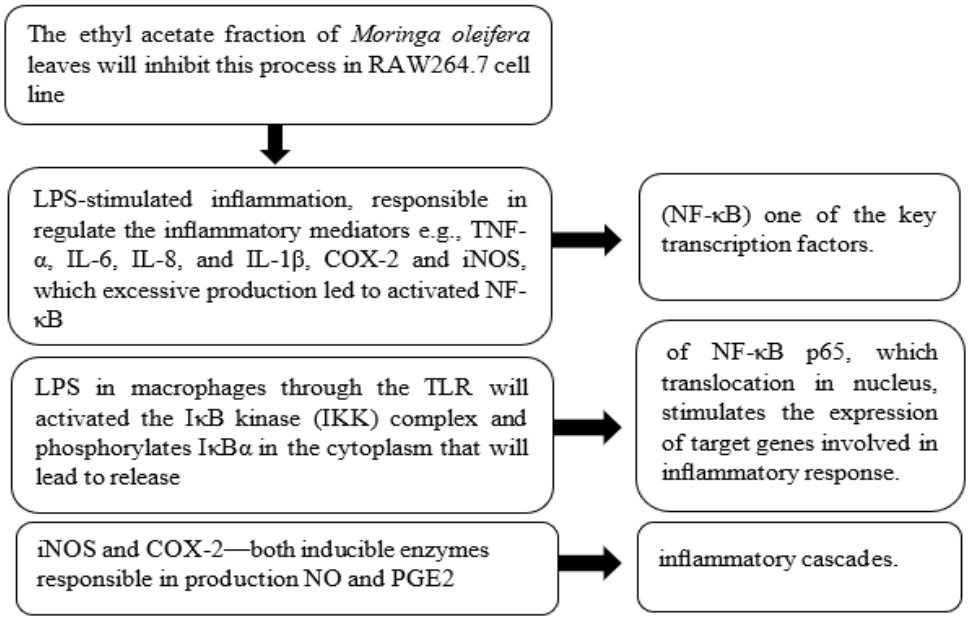

Figure 3: Flowchart of the strategy the ethyl acetate fraction of *Moringa oleifera* leaves in inhibition inflammatory process in RAW264.7 cell line(23)

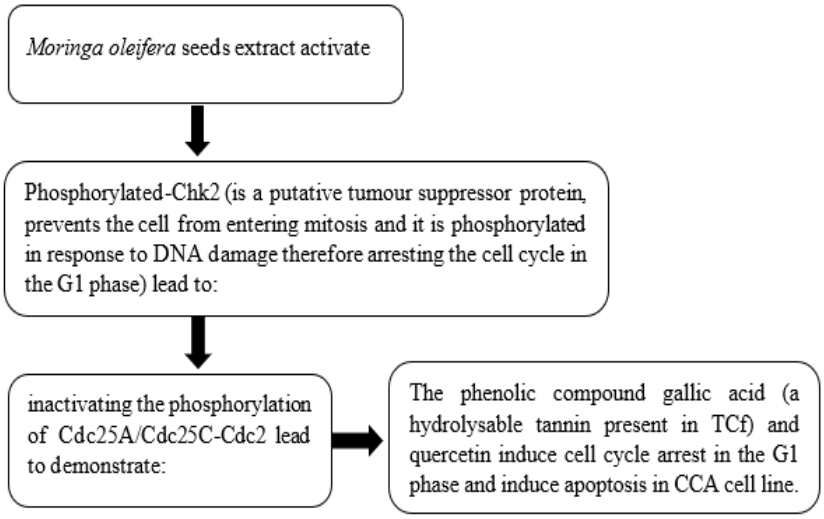

Figure 4: Flowchart of the strategy of *Moringa oleifera* seeds extract in induce apoptosis in CCA cell line(24)

Induction of apoptosis on Cholangiocarcinoma cell (CCA)

Moringa oleifera seeds extract demonstrated anti-proliferative effects in Cholangiocarcinoma CCA cell line by controlling signal transduction molecules and promoting pro-apoptotic signals by demonstrating the activation of phosphorylated-p53, the expression level of cleaved-PARP (a DNA repair enzyme that is triggered in response to DNA strand breaks) was suggestively raised, Increased activation of caspase-3, and Phosphorylated-Chk2 (is a putative tumour suppressor protein), prevents the cell from entering mitosis and phosphorylated in response to DNA damage. Significantly increased of phosphorylated-p38 MAPK and phosphorylated-AKT was also achieved in this study (24). Flowchart in Figure 4 depicted this finding.

Induction of apoptosis on Human Epithelial Carcinoma cells (KB)

Moringa oleifera leaves is rich in phenolics such as kaempferol and quercetin. These two compounds play an important role in cell proliferation of KB cells through a dose-dependent inhibition manner. This was supported by the displayed result that showed the morphological modification and DNA fragmentation, induction of apoptosis and ROS (reactive oxygen species) production. These findings significantly portrayed that the plant may contribute as a cancer therapeutic agent (25).

Induction of apoptosis on Human Neuroblastoma cell (SH-SY5Y)

Moringin extract from *Moringa oleifera* seeds has an inhibiting effect on the nuclear translocation of NF-κB via time and concentration-dependent manner on tumour derived from SH-SY5Y human neuroblastoma cell lines. Morigin could stimulate apoptotic process through decreasing that in the G1 phase and increasing the cell population in both G2 and S phases. Apart from that, the expression of p21, p53, and Bax were raised at both the transcriptional level and protein, this compound has expressively improved the gene expression of both caspase 9 and 3 thus establishing an intrinsic apoptotic pathway. The findings indicated that Moringin from *Moringa oleifera* seeds again has a valuable promising role as an anticancer medication (26).

Induction of apoptosis on Human Prostate Cancer cell (PC-3)

Gucomoringin-isothiocyanate from *Moringa oleifera* dried pods have an anti-proliferative effect on human prostate adenocarcinoma cells PC-3 in a time-dependent way. This was proved by the proliferative inhibition and apoptotic induction in the cancer cell. Thus, can be claimed as a potent cancer therapeutic agent (27).

Induction of apoptosis on Gastric Cancer cell (GC)

The effect of *Moringa oleifera* dried seeds extract on the gastric cancer cell line was by achieved by enlarging the NDRG1metastatic suppressor (a key regulator in tumour progression- related signaling pathways). A study also determined that this plant can also be used it the treatment of GC metastasis, through inverting the EMT (epithelial-mesenchymal transition, causing polarized epithelial cells to miss cell-cell links). The ability of *Moringa* in the up-regulated NDRG1 bona fide metastasis suppressor was also shown in their study (28).

Induction of apoptosis on Ehrlich-Lettre ascites carcinoma cells (EAC)

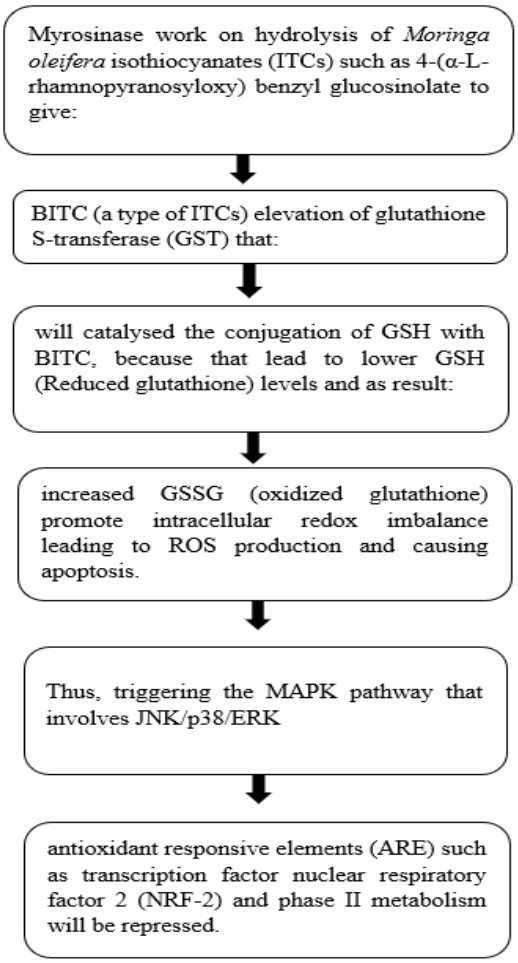

Figure 5: Flowchart of the strategy of *Moringa oleifera* in MCF-7 cell line(31)

Induction of apoptosis on Human ileocecal adenocarcinoma cell (HCT-8) and theTtriple-negative Breast Cancer cells (MDA-MB-231)

Moringa oleifera leaves and bark extracts on HCT-8 MDA-MB-231 cells line showed a significant increase in apoptosis in addition to G2/M improvement. The inhibition of focal adhesion kinase (p-FAK) or reduction of PI3K/AKT proteins target expression was also shown in their study. The findings clearly indicated that leaves and bark extracts from *Moringa oleifera* has promising role as an anticancer medication(30).

Induction of apoptosis on Breast Cancer cell line (MCF-7)

Moringa oleifera seeds displayed significant cytotoxic activity effect against breast adenocarcinoma (MCF-7) cells through p38-MAPK protein pathway (31). Flowchart strategy of *Moringa oleifera* seeds in MCF-7 cell line is depicted in figure 5.

A study done by Gaffar, Apriani and Herlina, 2019 has *Moringa oleifera* seeds lectin in other study has an inhibitory effect of 71.08 per cent at a concentration of 200 µg/ml against EAC cells through in a dose-dependent manner. The molecular pathway revealed was by caspase-3 inhibitor, Bak activation, suppression of Bcl-2 and NF-kB gene expression. All those are well known protein that plays a crucial role in apoptosis. Thus, this finding has prominently suggested lectin effect as an cancer therapeutic agent (29).

demonstrated the potential of *Moringa oleifera* leaves n-hexane fraction in inducing apoptosis and cell cycle arrest in the first, second growth and mitosis phase (G0-G1 and G2-M phase) against T47D breast cancer cells. This involved Bcl-2 anti-apoptosis protein and cell cycle controller protein, cyclin D1. They also showed the potential of the fraction in inducing apoptosis via p53-independent pathway in concentration-dependent manner(32). A similar study by Adebayo et al. (2019), showed that *Moringa oleifera* hexane fraction of the seeds (HF-CEE) exhibit antiproliferative properties in human breast cancer MCF7 cells. This has been proved by the morphological features of dead cells in tumour cell and not affecting normal breast cells, MCF 10A cell. Cell cycle result displayed that it arrested at G2/M and S phase as well. This happened via the controlling process of a variety cancer-associated protein such as HSP60, PDIA1, NPM, RCN1 and PGK1. These proteins are implicated in glycolysis, metastasis and invasive cancer (33). Another study illustrated that *Moringa oleifera* leaves n-hexane fraction (NHF) and ethanolic extract (EE) has a cytotoxic effect and apoptosis on MCF-7, human breast cancer lines, so that it can function as a chemotherapeutic agent for breast cancer, while molecular mechanism needs to be investigated (34). Adebayo IA et al. (2017), found that phytochemicals derived from *Moringa oleifera* seeds have been showed to have an antiproliferative effect on MCF-7. However, the mechanism of action and signaling pathways are required to be determined in order to complete the finding (35).

A study by Ghosh et al. (2013) has determined the wound healing properties in *Moringa oleifera* leaves. The plant extract has shown to potently inhibited the cellular adherence in a dose-dependent and time dependent manner towards MCF 7 and MDA MB 231 with less cytotoxicity in the normal cells (36). Hossain, Mirghani and Raus et al. (2015), showed that extracted leaves of *Moringa oleifera* inhibit the proliferative of human breast cancer MCF-7 cell line with an average of 87.13 per cent at wavelength A570 nm. Thus, proved that it profound a significant role in the treatment of this type of cancer (37). Another similar study by Poobalan et al. (2018), showed that dry leaf powder samples of *Moringa oleifera* leaves demonstrated anticancer effects against human breast adenocarcinoma cell line, MCF-7, and no cytotoxicity

responses on the non-tumour cell line, MCF-10A. As a result, it can be used alone or in conjunction with chemotherapeutic for breast cancer treatment (38). Chen G et al. (2014) in their study showed that by using the extracts of *Moringa oleifera* root bark at 50 g/mL, 56 per cent of cells were reduced against MCF-7/ADR tumour cell lines (39). A recent study by Taha et al. (2019), found that *Moringa oleifera* roots extracts with different concentrations; 5, 12.5, 25, 50 µg/ml were used on MCF7 cell line and displayed a strong cytotoxicity result. While the mechanism of action, and signaling pathways are required to be explored (40). The findings based on all *in vitro* studies on MCF7 cell line, obviously confirmed that this plant targeting multiple pathways which contribute to its anti-cancer properties.

Induction of apoptosis on normal human keratinocyte (HaCaT) and the human melanoma cell line (A2058)

Moringa oleifera fruit extract indicated an important role in suppression of the cell viability and confirmed apoptosis in A2058 cells in a dose-dependent manner. These increased caspase-3 and caspase-9 cleaved activities. Besides, MAPK (mitogen-activated protein kinases) phosphorylation and ROS (reactive oxygen species) production were also increased significantly (41). Flowchart strategy of *Moringa oleifera* fruit extract in A2058 HaCaT cell line is depicted in Figure 6.

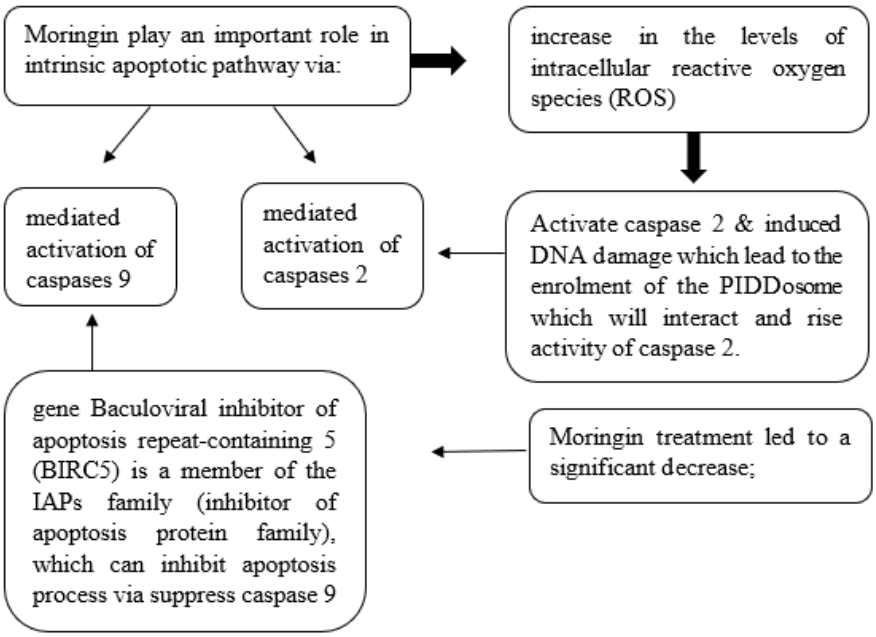

Figure 6: Flowchart of the strategy of *Moringa oleifera* fruit extract in A2058 HaCaT cell line (41)

Induction of apoptosis on human liver adenocarcinoma cell (Hep3B)

Antonini et al. (2018), incorporated and demonstrated that Moringin of *Moringa oleifera* seeds with Avenanthramide 2f (phytochemical extracted from oats) can prevent the proliferation of Hep3B Liver Cancer Cells, via the increasing activity of caspases 8, 2, 3, and 9. Moringin portrayed intrinsic apoptotic pathway by significantly increase in the levels of intracellular reactive oxygen species (ROS), mediated activation caspase 2, 9 and downregulation of the prosurvival gene BIRC5. While extrinsic pathway done by AVN 2f mediated activation caspase 8. The findings indicated that Moringin of *Moringa oleifera* seeds alone or with Avenanthramide 2f has promising role as an anticancer medication (42). Flowchart strategy of *Moringa oleifera* in intrinsic apoptotic pathway is depicted in Figure 7.

Induction of apoptosis on Hepatic stellate cells (Hepalclc7)

Tumer et al. (2015), showed the strategy of *Moringa oleifera* leaves extract in hepatoma Hepalclc7 cells, by isothiocyanate (ITC) and polyphenol, which were extracted and fractionate by centrifugal partition chromatography (FCPC). Polyphenols have direct antioxidant activity and isothiocyanate (ITC) that have indirect antioxidant activity through encouraged NQO1(cytoprotective marker enzyme) activity. The findings indicated that leaves extract from *Moringa oleifera* has promising role as an anticancer medication (43). Flowchart strategy of *Moringa oleifera* leaves extract in hepatoma Hepalclc7 cells is depicted in Figure 8.

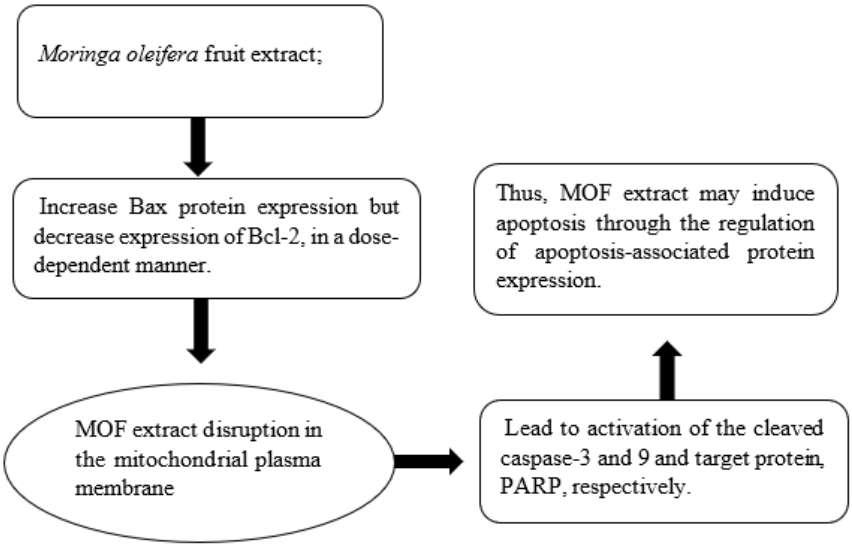

Figure 7: Flowchart of the strategy of *Moringa oleifera* in intrinsic apoptotic pathway (42)

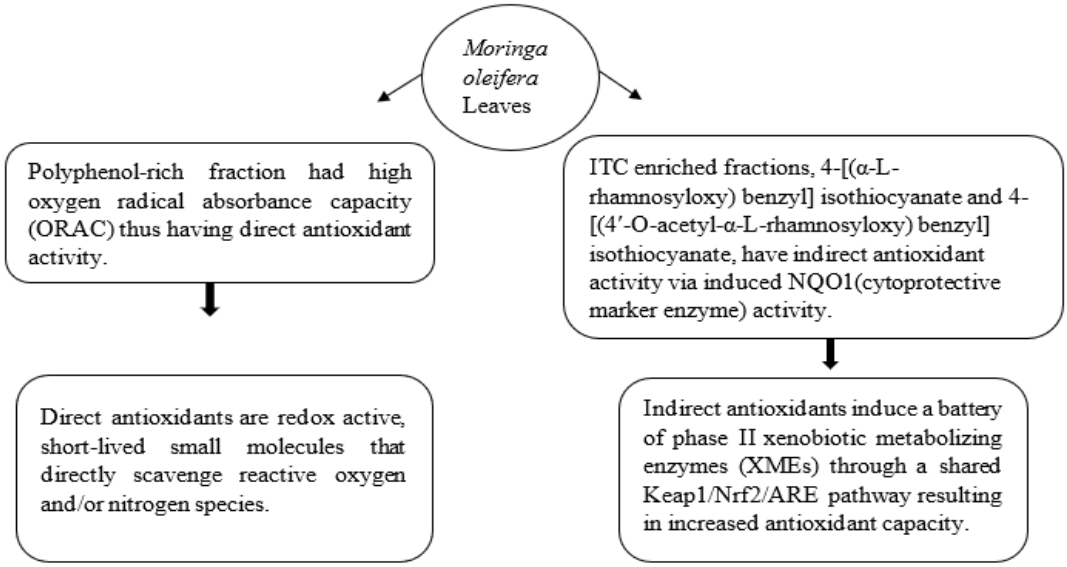

Figure 8: Flowchart of the strategy of *Moringa oleifera* leaves extract in hepatoma Hepalclc7 cells (43)

Molecular mechanisms involved in in vivo studies.

In the first *in vivo* study, two tumour cell lines, A549 and HepG2, were cultured in 75-cm² culture flasks. Surgical implantation was carried out in 6-week-old male immunodeficient nude mice and were orally administered with *Moringa oleifera* leaf extract in different concentrations. After seven days, the fibers were collected and exposed to the trypan blue exclusion assay. *Moringa oleifera* leaf extracts with higher concentrations prevented cell propagation by downregulating the anti-apoptotic Bcl-xL protein and boosting cleaved caspase-3 and cleaved PARP expression markers for apoptotic process. A stately decrease in cell viability was observed for the HepG2 cells, whereas same kinetic profile was achieved for the A549 cells (6). In contrary, Hagoel et al. (2019), in their study utilized PANC-1 cancer cells which were injected subcutaneously into the flank area. Immune deficient athymic CD-1 nude mice were used for a xenograft ectopic tumour modelling in the other selected study. The 6- to 7-week-old females' mice were injected intraperitoneally two times weekly for six weeks by *Moringa oleifera* Lam Leaves (0.5,1.0, and 1.5 mg/g, 200 micro-L/ mouse). Result showed that the tumour volume at the end of the experiment was smaller. *Moringa oleifera* leaf extracts inhibit pancreatic tumour growing in Nude Mice via dose-dependent manner and confirmed antiangiogenic motion and antiproliferative effect in tumours due to the Ki-67 Immunohistochemical analysis, which showed that

Moringa extracts repressed tumour development, but difference did not reach a level of significance between the groups treated with *Moringa* and without it. *Moringa* also reduced the expression of both proteins IκB-α, and the NF-κB inhibitor, and effect on the expression of anti-apoptotic Bcl-2 proteins (11).

A recent study by Jaafaru et al. (2018), demonstrated the ability of Gucomoringin-isothiocyanate rich soluble extracts (GMG-ITC-RSE) pre-filtered water was dosed orally into dried pods of *Moringa oleifera*. Female Sprague-Dawley (SD) Inbreed-specific pathogen-free (SPF) rats weighing 249.94 g in the test group, 232.36 g in the ongoing test group, and 260.18 g in the control group. For test groups, the dose was in 10 mL/kg, while control animals were given 10 mL/kg daily for 14 days in a parallel way, the resulting proven acute toxicity coupled with a high-security in vitro anti-tumour activity (27). A study by Cuellar-Nuñez et al. (2018), was carried out in four groups of male CD-1 mice, each group have six mice (colorectal carcinogenesis model). Group one (negative control/NC) was fed with basal diet, group two (positive control) received AOM/DSS, group three was fed basal diet complemented with *Moringa oleifera* leaves (2.5 % w/w), and group four also was fed with basal diet complemented with *Moringa oleifera* leaves (5 % w/w). The time duration was twelve weeks. This research possesses that *Moringa oleifera* leaf contains nine phenolic compounds namely coumaric and chlorogenic acid. Particularly insoluble and soluble dietary fibre. It also displayed chemo-preventive measurements by decreased effect on the pro-inflammatory cytokines including (IL-6 and IL-12) in colonic macrophages. Decreased activity of fecal harmful enzymes like as β -glucosidase and β-glucuronidase, tryptophanase and urease were observed (44). The findings indicated that *Moringa oleifera* leaves has promising role as an anticancer property. Flowchart strategy of *Moringa oleifera in vivo* (colorectal carcinogenesis model) study is depicted in Figures 9, 10, 11**.**

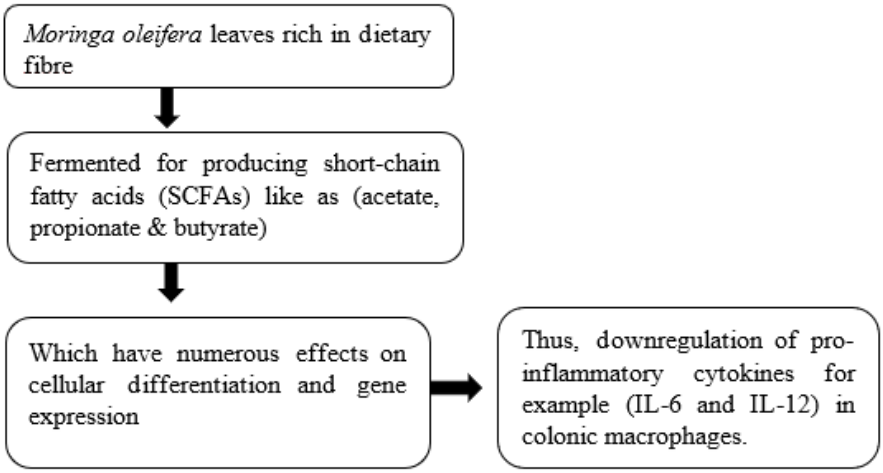

Figure 9: Flowchart of the strategy of *Moringa oleifera in vivo* (colorectal carcinogenesis model) study (44)

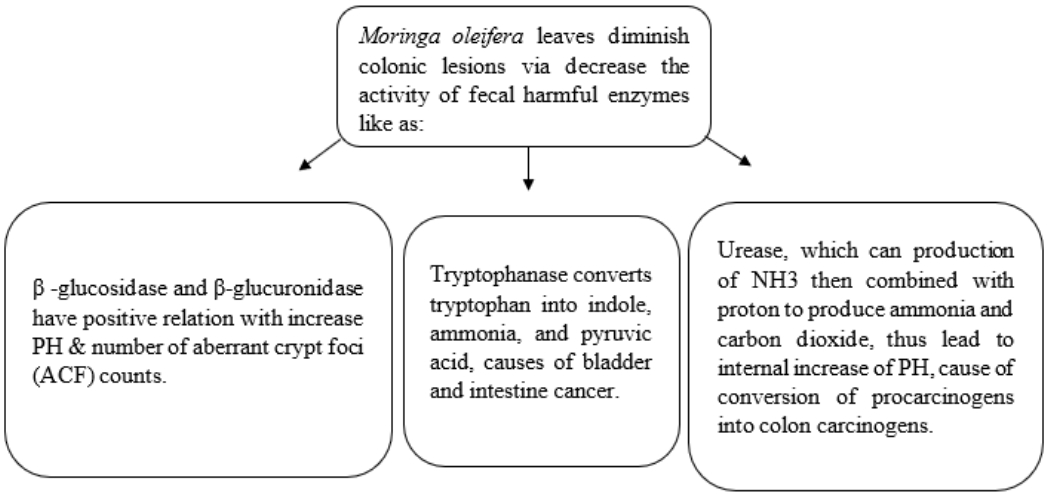

Figure 10: Flowchart strategy of *Moringa oleifera in vivo* (colorectal carcinogenesis model) study (44)

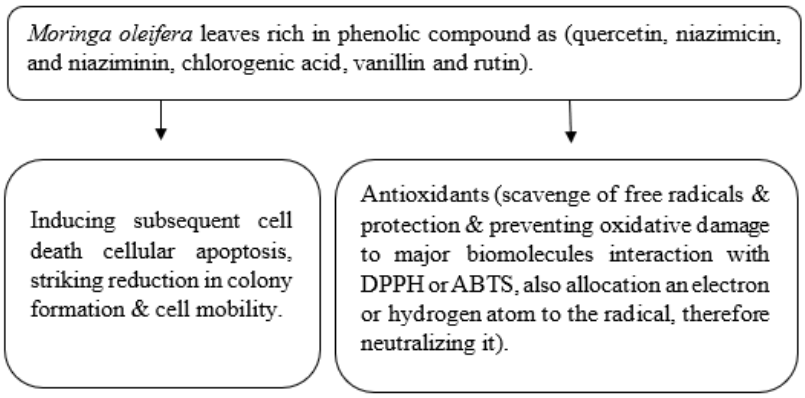

Figure 11: Flowchart strategy of *Moringa oleifera in vivo* (colorectal carcinogenesis model) study (44)

Kraiphet et al. (2018) carried out an *in vivo* study in boiled *Moringa oleifera* pods was. Four groups or mice were used. Each group have eight mice (Three-week-old male ICR mice (Mus musculus), weighted 15 ± 3 g) induced by azoxymethane (AOM, 10 mg/ kg BW i.p. at the third week). Later those mice were induced by dextran sodium sulphate (DSS) for seven days with one negative group induced by AIN-76A. Basal diet starved from AOM/DSS with one negative group induced by AOM/DSS, while AOM/DSS induced the healthy control group. The treatment group received basal diet containing 1.5 per cent, 3.0 per cent, and 6.0 per cent of boiled *Moringa oleifera* pods at week number five and continue until 20 weeks after the induction of AOM/DSS.

The effect has been shown to induce of apoptosis in colon cancer by differentiating of BCL-2 family protein expression and reducing the expression of BCL-2 protein. BAX protein that has the ability to facilitate cell death was also upregulated. The upregulated of BAX/BCL-2 ratio is related to an intrinsic pathway. Thus, this is a proof to its inhibitory effect on colon cancer (45). Flowchart strategy of *Moringa oleifera in vivo* (colon cancer) study is depicted in Figure 12.

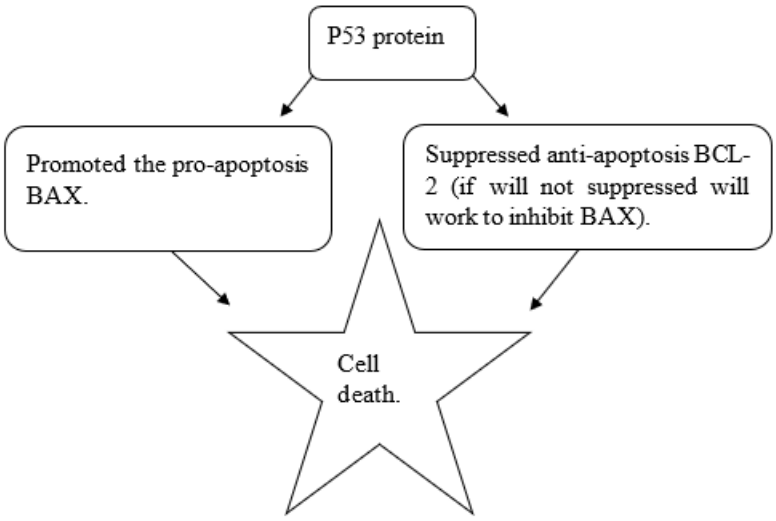

Figure 12: Flowchart strategy of *Moringa oleifera in vivo* (colon cancer) study (45)

Other study which utilized lectin of *Moringa oleifera* seeds was injected (i.p.) in EAC adult Swiss albino mice (two months old and the average weight with 27 g) the dose was 2 & 4 mg/kg/day for 5 days, and the cell growth inhibition was 25.38 per cent and 55 per cent, respectively. The inhibition in concentration-dependent pattern for cell growth and cell cycle was demonstrated by FACS flow cytometry and was arrested at G2/M

phase. Condensed nuclei and irregular shape of cells was observed. Apoptotic proteins which is Caspase-3 has been activated via interaction with different protein. The inhibitor (z-DEVD-fmk) was blocking the binding sites on caspase-3 as a result after treatment of EAC cells with lectin, thus prove the potential of the plant I the reduction of cell proliferative in EAC cells. Inhibition of NF-kB genes expression and Bcl-2 together with the stimulation of Bak. This is clearly correlated with the anticancer properties of purified *Moringa oleifera* seed lectin (MOSL) (29). According to study published by Sadek et al. (2017), *Moringa oleifera* leaf ethanol extract (MOLEE) was gastrogavaged in Wistar rats (weighing 140–160g) (500mg/kg) for seven days then MOLEE for 16 weeks with Diethyl nitrosamine (DEN), a natural cancer-causing agent's as appositive control. The researchers have been used to evaluate the prophylactic impacts. Histological components such as oxidation of DNA and serum biomarker of the liver tissues. The robotic studies set that MOLEE reduced the DEN-activated oxidative reactivity damage in rats by 46.8 per cent. Result showed the ability of MOLEE to reduce the expression of β-arrestin-2, Bcl-2 and Bcl-xl, with ($p<0.05$). However, the expression was essentially upregulated caspase 3, and Bax, with ($p<0.05$). Interestingly, MOLEE exhibit the enactment of antioxidant activity and apoptosis by important defensive effects when compared to the DEN-induced hepatocarcinogenesis.

Thus, MOLEE indicated a promising chemoprevention against Hepatocellular carcinoma HCC (46). Flowchart strategy of *Moringa oleifera in vivo* (Hepatocellular carcinoma HCC) study is depicted in Figure 13.

Based on all *in vivo* studies, *Moringa oleifera* leaves, pods, dried pods and seeds, has significant effect on the molecular pathways which may lead to anti-cancer event. This pathway includes the prevention of cell propagation by the downregulated of anti-apoptotic (Bcl-xL) protein, boosting cleaved in caspase-3 and cleaved PARP expression markers for apoptotic process. Thus, this may indicate the antiangiogenic and antiproliferative effect in tumors due to the Ki-67 Immunohistochemical analysis. Apart from that, it also downregulates the expression of both proteins IκB-α, and the NF-κB inhibitor while very much affecting on the expression of anti-apoptotic Bcl-2 proteins. In addition, insoluble and soluble dietary fibre, have shown chemo-preventive measurements by the downregulation of pro-inflammatory cytokines for example (IL-6 and IL-12) in colonic macrophages. The decrease activity of fecal harmful enzymes like as β -glucosidase and β-glucuronidase, tryptophanase and urease was also seen in these findings. Thus, *Moringa oleifera* leaf ethanol extract (MOLEE) treatment could potentially decreased β-arrestin-2 mRNA expression levels and portrayed an apoptotic signaling, as proven by the upregulation of Bax and caspase-3. Therefore, by referring to its anticancer properties, the biomedical and pharmaceutical industries could use it as anticancer herbal medicine.

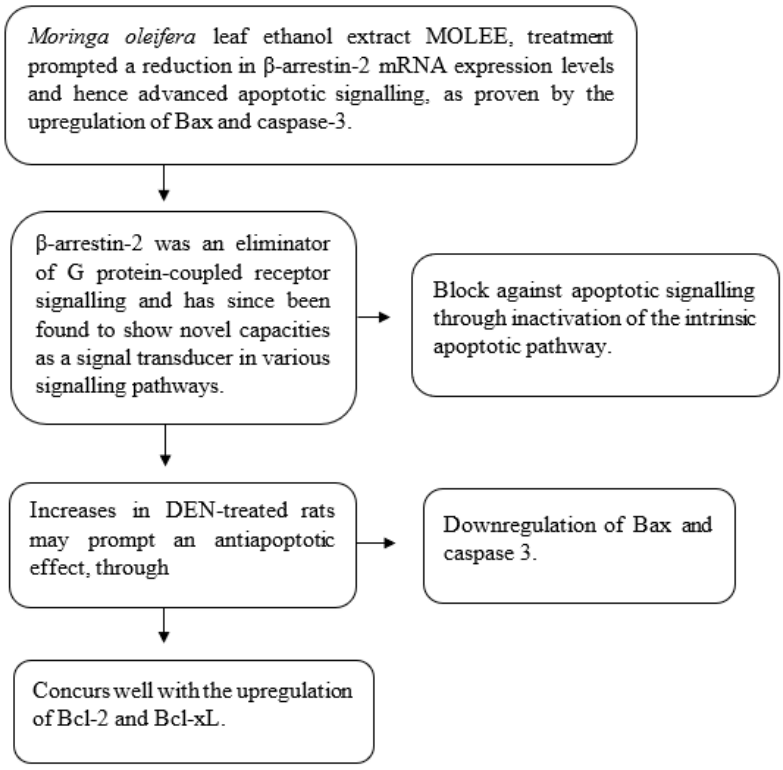

Figure 13: Flowchart strategy of *Moringa oleifera in vivo* (Hepatocellular carcinoma HCC) study (46)

Cell cycle analysis on Moringa oleifera targeted protein pathways involved.

The capability to control cell apoptosis becomes the key for immense therapeutic potential. Thus, many studies focus on emphasizing the elucidation and analysis of the cell cycle machinery and signaling pathways that control cell cycle arrest and apoptosis particularly after treatment with potential anticancer compounds (25). Macrophages, which are responsible for initiating and modulation of hosts, describe mechanisms by producing of proinflammatory mediators such as (cytokines, prostaglandin E2 (PGE2) and nitric oxide) (23). Morphological changes in apoptotic cells triggered by membrane blebbing. It includes many changes such as a loss of contact with neighboring cells and cytoplasmic membrane shrinkage under the control of gene regulation (25). Mitochondria are a very important part in the cell, which its responsible for the execution of cell death and regulate energy-producing, as well as paly an important role in producing reactive oxygen and nitrogen species, they are also linked to intracellular death signaling, cell proliferation, and disease pathogenesis (14). During apoptosis, mitochondria produce ROS (reactive oxygen species), and when the membrane reduces, it led to the excess generation of apoptosis, and ROS which contributed a second's messenger in several signaling pathways, this phenomenon plays an important role in regulating the action of specific enzymes implicated in the cell death pathway (25). Reactive oxygen species (ROS) stimulate aging and cancer, proteins and nucleic acids destruction, lipid peroxides formation and the suppression of various enzyme function by attacking living cells. ROS are also intracellular signaling mediators. With increased, production of ROS lead to increased oxidative stress, cellular damage will occur, and cellular functions inhibition and the cell cycle apoptosis will occur (9). Caspase-3 protease is activated in apoptotic cells by intrinsic (mitochondrial) pathways and extrinsic (death ligand) pathways and applies to pro-apoptotic roles through the cleavage of multiple cellular targets, plus inhibiting PARP (a DNA repair enzyme that is activated in response to DNA strand breaks) function. Also inducing accumulation of ROS (reactive oxygen species) and mitochondrial dysfunction lead to apoptosis cancer cells (24). Abd-Rabou et al. (2017), showed that *Moringa* extract prevents the arrest G1 cell cycle phase and cancer cells proliferation via special thioredoxin interacting protein (TXNIP) by p27kip1 protein. While showing less toxic on normal cells(20). Current study by Cirmi et al. (2019) reported that moringin extract from *Moringa oleifera* seeds play a major important role in cell cycle arrest thereby stimulating p53, regulates and stimulates p21 thus responsible for the cyclin-dependent kinase inhibitory (CDKI) protein. This will then affect the affinity complexes G1 and G2 cyclin-CDK (26).

A study by Reda et al. (2017), demonstrated that phytochemicals, for example glucosinolates (GLs) and their breakdown products (i.e., ITCs as benzyl ITC, allyl ITC, phenethyl ITC, and sulforaphane) were subjected to avert the risk of carcinogenesis (10). ITCs are a category of natural products which produced and released myrosinase enzymatic action on their GLs precursors after cell damage (26). ITCs exhibit cell cycle arrest, apoptosis, and metastasis prevention. ITCs cause acute oxidative cellular stress, depolarization of the mitochondria, and cell damage. Tiloke et al. (2019), summarized the strategy of the crude aqueous extract from *Moringa oleifera* leaves in cell cycle process through the upregulated of numerous molecule such as the pro-apoptotic protein Bax, Smac/DIABLO protein expression (responsible for binds inhibitor of apoptosis proteins, therefore freeing caspases to trigger apoptosis), levels of reactive oxygen species (ROS). SRp30a protein (Serine and Arginine Rich Splicing Factor 1 [SRSF1]) work as a central regulator of gene expression and cellular homeostasis) expression. While decreased c-myc (important regulator of the cell cycle) and anti-apoptotic protein Bcl2, plus significantly reduced cells in the G1 phase, S phase and G2-M phase. Decrease ATP level on the other hand may lead to the activation of caspase 9 (an initiator caspase for the intrinsic apoptotic pathway)(7). Flowchart strategy of *Moringa oleifera* in cell cycle process is depicted in Figure 14.

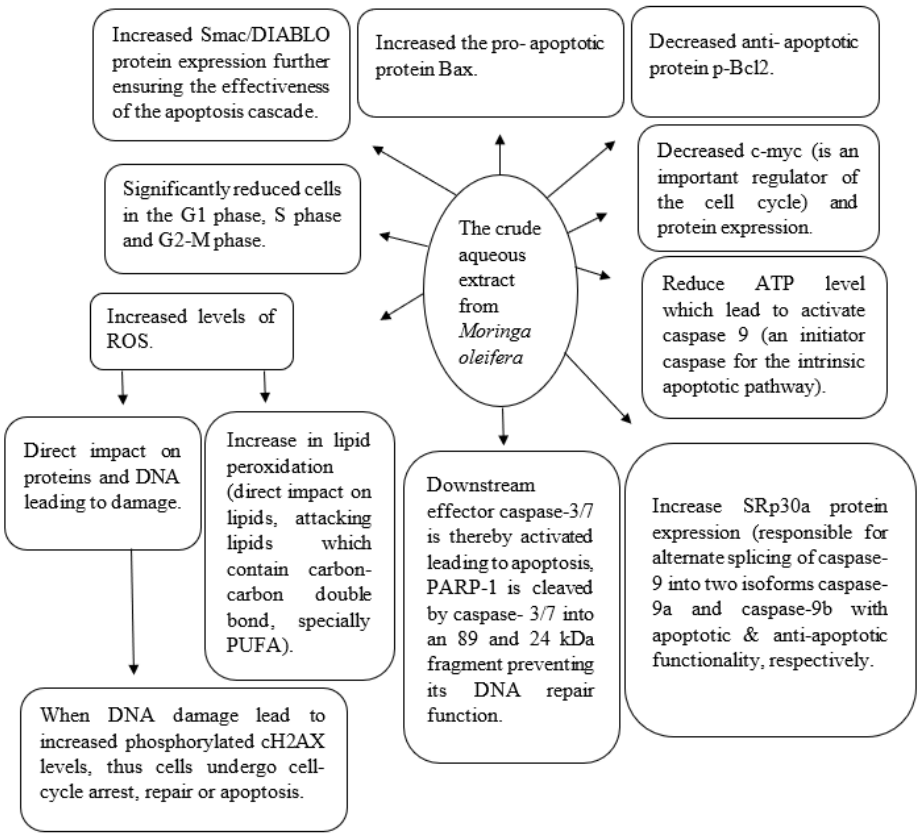

Figure 14: Flowchart strategy of *Moringa oleifera* leaves in cell cycle process (7)

A study by Leelawat et al. (2017), found that tumour protein p53 (is a gene that codes for a protein that controls the cell cycle) has a vital function in cell apoptosis during accumulation, the phosphorylation of p53 at Ser15 promotes the efficient start and accumulation of p53 in response to DNA damage or apoptotic stimulation by stimulating protein translocation to the mitochondria (intrinsic pathway), thus prompt apoptosis through (mitochondrial) pathways (24). Comparable study by Kraiphet et al. (2018), showed that tumour protein p53 plays a major role in apoptosis via intrinsic pathway, which in effect promotes pro-apoptosis BAX protein, on the other hand, suppressing the anti-apoptosis BCL-2 protein, culminating in cell death (45). Flowchart strategy of *Moringa oleifera* in targeted protein pathway is depicted in Figure 15.

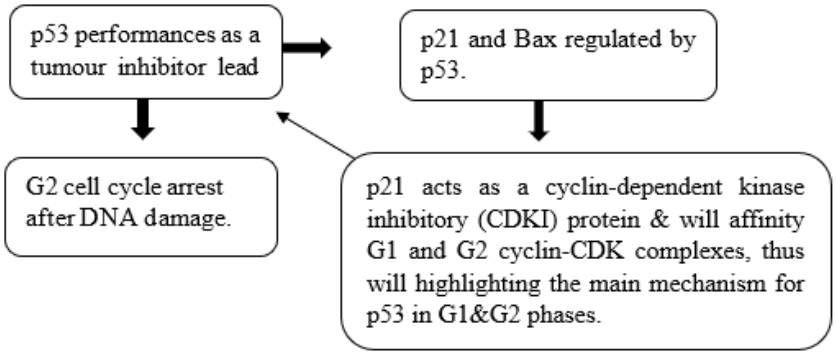

Figure 15: Flowchart strategy of *Moringa oleifera* in targeted protein pathway (26)

Michl et al. (2016) reported that moringin (GMG-ITC) from *Moringa oleifera* seeds inhibits the signaling pathway of JAK/STAT through preventing of IL-3-induced STAT5 (Signal Transducer and Activator of transcription 5) signaling at concentrations of 0.4 µM. The study also displayed the downregulation abilities of the seed extracts in IFNα-induced STAT1, STAT2 activity and TNF-induced NF- κB signaling(47). Guon and Chung et al. (2017), in their study demonstrated that *Moringa oleifera* fruit extract activate MAPKs (a mitogen-activated protein kinase). This involved in directing cellular responses to numerous stimuli and consist of ERK protein (is involved in cell proliferation), JNK and p38 proteins (encompassed in proliferation inhibition and apoptosis)(9). Flowchart of *Moringa oleifera* in targeted proteins pathways is depicted in Figure 16.

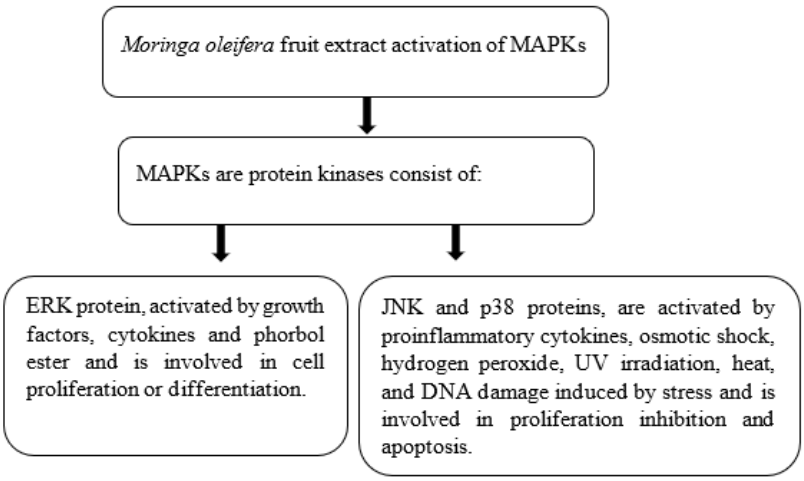

Figure 16: Flowchart strategy of *Moringa oleifera* in targeted proteins pathways (9)

Adebayo et al. (2019), showed that the hexane fraction of the seeds (HF-CEE). The upregulated of these three proteins (tubulin βII t, NPM, and hnRNPF protein) were showed to be very important to prove the anti-cancer properties of this plant. Apart from those seven proteins (TPM3, PKM2, PDIA1, RCN1, HSP60, PGK1 and K6 protein) in human breast cancer MCF7 cells were downregulated (33). Flowchart showed the hexane fraction of *Moringa oleifera* seeds (HF-CEE) in targeted protein pathway in CF7 cells Figure 17.

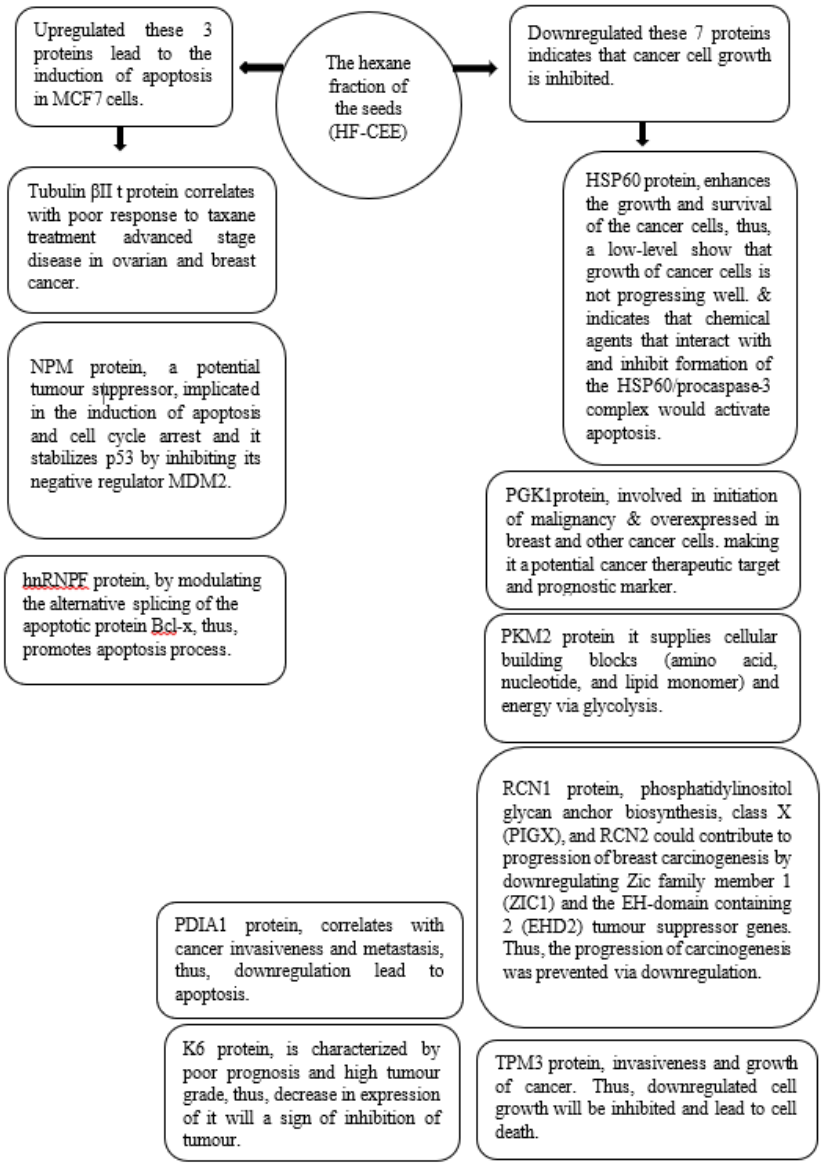

Figure 17: Flowchart strategy of *Moringa oleifera* seeds in targeted protein pathway in MCF7 cells (33)

The anticancer potential and molecular targets of Moringa oleifera

Moringa oleifera can target numerous molecules and proteins to suppress cancer cell progression. Previous studies proved that *Moringa oleifera* possess the ability to enhance or increase the activity of some molecules such as, activation of caspase-9 and caspase-3, and the cleaved form of PAPR (poly ADP-ribose polymerase). Boost of p53, p53 mRNA, p21WAF1/Cip1 and p27Kip1 protein levels, proapoptotic protein Bax, p21, and activation of PARP-1 into 89 KDa and 24 KDa fragments, plus boost mitochondrial ROS (reactive oxygen species) producing, increased expression levels of phosphorylated-p38 MAPK and phosphorylated-AKT.

While decrease or inhibit other molecules such as, ERK1/2 phosphorylation, reduced expression of the pro-apoptotic protein Bcl-2, downregulated the key component of DNA repair pathways PARP-1, COX-2 [apoptosis-related proteins], p65-subuni, and decreased the expression of IkBα, p65 and p-IkBα proteins (all three proteins of the NF-κB signaling pathway), decrease in Nrf2 protein, sirtuin-1 (SIRT1) protein expression. exhibited an inhibitory effect on CYP3A4 which is one of major drug-metabolizing Cytochrome P450 enzymes, suppressing the expression of inflammatory mediators including inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2. Flowchart twenty molecular target of *Moringa oleifera* is depicted in Figure 18. Therefore, *Moringa oleifera* has the potential in the evolution of complementary therapeutic medicine versus cancer.

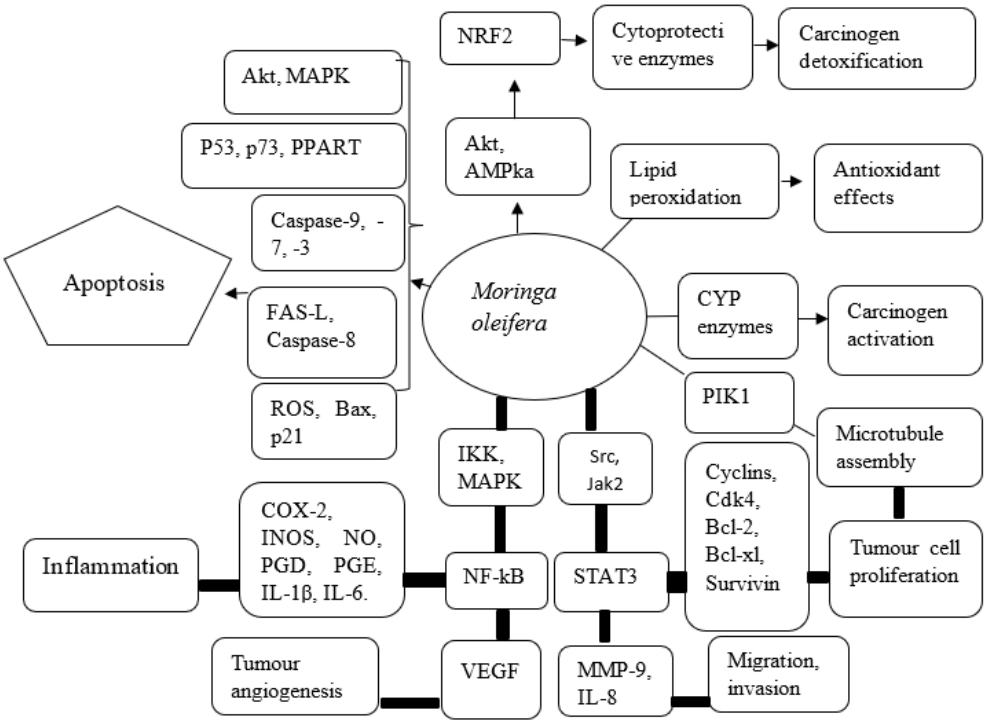

Figure 18: Twenty molecular targets of *Moringa oleifera* (48)

Akt, Protein kinase B; AMPkα, 5' adenosine monophosphate-activated protein kinase α; NRF2, nuclear factor (erythroid-derived 2)-like 2; CYP, Cytochrome P450; PIK1, Phosphatidylinositol 4-kinase; Src, Proto-oncogene tyrosine-protein kinase; Jak 2, Janus kinase 2; STAT3, Signal transducer and activator of transcription 3; MMP-9, Matrix metallopeptidase 9; IL-8, Interleukin 8; IKK, IκB kinase; MAPK, mitogen-activated protein kinases; NFκB, nuclear factor kappa B; VEGF, Vascular endothelial growth factor; COX-2, cycloox-ygenase-2; INOS, Inducible NOS; PGD, prostaglandin D; PGE, prostaglandin E; IL-1β, Interleukin 1β; IL-6, Interleukin 6; P53, Tumour suppressor protein p53; p73, Tumour suppressor protein p73; PPARγ, Peroxisome proliferator-activated receptor γ; Cas- pase-9, -7, -3, cysteine-aspartic proteases -9 (initiator), -7 (executioner), -3 (executioner); FAS-L, FAS ligand; Caspase-8, cysteine- aspartic proteases 8 (initiator); ROS, reactive oxygen species; Bax, Bcl-2-associated X protein; p21, Cyclin-dependent kinase inhibitor.

Conclusion

Studies presented in this review revealed Moringa oleifera as one of the potential medicinal plants in preventing and treating cancer, as demonstrated by *in vitro* and *in vivo* testing. Polyphenols have been identified as one of the most widely used natural anti-cancer compounds. The elimination of carcinogenic agents, modulation of cancer cell signalling and antioxidant enzymatic activities, and induction of apoptosis are the most likely approaches to dietary polyphenol anticancer effects.

However, the reviewed literature has several limitations. More research is needed to determine the active compound threshold value of *Moringa oleifera*. Estimation of the number of polyphenols required to treat certain cancers effectively. More research is needed to confirm *Moringa oleifera's* toxicity.

Acknowledgements

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Competing interests

The authors declare there is no competing interest associated with this work.

Reference

1. Pangastuti A, Amin IF, Amin AZ, Amin M. Natural bioactive compound from Moringa oleiferaagainst cancer based on in silico screening. J Teknol. 2016;78(5):315–8.
2. Alves-Silva JM, Romane A, Efferth T, Salgueiro L. North African Medicinal plants traditionally used in cancer therapy. Front Pharmacol. 2017;8(JUN):1–24.
3. Fantoukh OI, Albadry MA, Parveen A, Hawwal MF,

Majrashi T, Ali Z, *et al*. Isolation, synthesis, and drug interaction potential of secondary metabolites derived from the leaves of miracle tree (Moringa oleifera) against CYP3A4 and CYP2D6 isozymes. Phytomedicine. 2019;60:153010.
4. Castro-Puyana M, Marina ML, Plaza M. Water as green extraction solvent: Principles and reasons for its use. Curr Opin Green Sustain Chem. 2017;5:31–6.
5. Nobossé P, Fombang EN, Mbofung CMF. Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh Moringa oleifera L. leaves. Food Sci Nutr. 2018;6(8):2188–98.
6. Jung IL, Lee JH, Kang SC. A potential oral anticancer drug candidate, Moringa oleifera leaf extract, induces the apoptosis of human hepatocellular carcinoma cells. Oncol Lett. 2015;10(3):1597–604.
7. Tiloke C, Phulukdaree A, Gengan RM, Chuturgoon AA. Moringa oleifera Aqueous Leaf Extract Induces Cell-Cycle Arrest and Apoptosis in Human Liver Hepatocellular Carcinoma Cells. Nutr Cancer. 2019;71(7):1165–74.
8. Tragulpakseerojn J, Yamaguchi N, Pamonsinlapatham P, Wetwitayaklung P, Yoneyama T, Ishikawa N, *et al*. Anti-proliferative effect of Moringa oleifera Lam (Moringaceae) leaf extract on human colon cancer HCT116 cell line. Trop J Pharm Res. 2017;16(2):371–8.
9. Guon TE, Chung HS. Induction of apoptosis with Moringa oleifera fruits in HCT116 human colon cancer cells via intrinsic pathway. Nat Prod Sci. 2017;23(4):227–34.
10. Reda F, Borjac J, Fakhouri R, Usta J. Cytotoxic effect of Moringa oleifera on colon cancer cell lines. Acta Hortic. 2017;1158:269–78.
11. Hagoel L, Vexler A, Kalich-Philosoph L, Earon G, Ron I, Shtabsky A, *et al*. Combined Effect of Moringa oleifera and Ionizing Radiation on Survival and Metastatic Activity of Pancreatic Cancer Cells. Integr Cancer Ther. 2019;18.
12. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. Moringa Oleifera aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complement Altern Med. 2013;13.
13. Gismondi A, Canuti L, Impei S, Di Marco G, Kenzo M, Colizzi V, *et al*. Antioxidant extracts of African medicinal plants induce cell cycle arrest and differentiation in B16F10 melanoma cells. Int J Oncol. 2013;43(3):956–64.
14. de Andrade Luz L, Rossato FA, Costa RAP e., Napoleão TH, Paiva PMG, Coelho LCBB. Cytotoxicity of the coagulant Moringa oleifera lectin (cMoL) to B16-F10 melanoma cells. Toxicol Vitr. 2017;44:94–9.
15. Tiloke C, Phulukdaree A, Anand K, Gengan RM, Chuturgoon AA. Moringa oleifera Gold Nanoparticles Modulate Oncogenes, Tumor Suppressor Genes, and Caspase-9 Splice Variants in A549 Cells. J Cell Biochem. 2016;13(July 2015):2302–14.
16. Rajan TS, De Nicola GR, Iori R, Rollin P, Bramanti P, Mazzon E. Anticancer activity of glucomoringin isothiocyanate in human malignant astrocytoma cells. Fitoterapia. 2016;110:1–7.
17. Vasanth K, Minakshi GC, Ilango K, Mohan Kumar R, Agrawal A, Dubey GP. Moringa oleifera attenuates the release of pro-inflammatory cytokines in lipopolysaccharide stimulated human monocytic cell line. Ind Crops Prod. 2015;77:44–50.
18. Potest M, Minutolo A, Gismondi A, Canuti L, Kenzo M, Roglia V, *et al*. Cytotoxic and apoptotic effects of different extracts of Moringaoleifera Lam on lymphoid and monocytoid cells. Exp Ther Med. 2019;5–17.
19. Ezhilarasi AA, Vijaya JJ, Kaviyarasu K, Maaza M, Ayeshamariam A, Kennedy LJ. Green synthesis of NiO nanoparticles using Moringa oleifera extract and their biomedical applications: Cytotoxicity effect of nanoparticles against HT-29 cancer cells. J Photochem Photobiol B Biol. 2016;164:352–60.
20. Abd-Rabou AA, Abdalla AM, Ali NA, Zoheir KMA. Moringa oleifera root induces cancer apoptosis more effectively than leave nanocomposites and its free counterpart. Asian Pacific J Cancer Prev. 2017;18(8):2141–9.
21. Elsayed EA, Sharaf-Eldin MA, Wadaan M. In vitro evaluation of cytotoxic activities of essential oil from Moringa oleifera seeds on HeLa, HepG2, MCF-7, CACO-2 and L929 cell lines. Asian Pacific J Cancer Prev. 2015;16(11):4671–5.
22. C. Maiyo F, Moodley R, Singh M. Cytotoxicity, Antioxidant and Apoptosis Studies of Quercetin-3-O Glucoside and 4-(?-D-Glucopyranosyl-1?4-?-L-Rhamnopyranosyloxy)-Benzyl Isothiocyanate from Moringa oleifera. Anticancer Agents Med Chem. 2015;16(5):648–56.
23. Arulselvan P, Tan WS, Gothai S, Muniandy K, Fakurazi S, Mohd Esa N, *et al*. Anti-inflammatory potential of ethyl acetate fraction of moringa oleifera in downregulating the NF-κB signaling pathway in lipopolysaccharide-stimulated macrophages. Molecules. 2016;21(11):1–13.
24. Leelawat S, Leelawat K. Molecular mechanisms of cholangiocarcinoma cell inhibition by medicinal plants. Oncol Lett. 2017;13(2):961–6.
25. Sreelatha S, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by Moringa oleifera leaf extract on human cancer cells. Food Chem Toxicol. 2011;49(6):1270–5.
26. Cirmi S, Ferlazzo N, Gugliandolo A, Musumeci L, Mazzon E, Bramanti A, *et al*. Moringin from moringa oleifera seeds inhibits growth, arrests cell-cycle, and induces apoptosis of SH-SY5Y human neuroblastoma cells through the modulation of NF-kB and apoptotic related factors. Int J Mol Sci. 2019;20(8).
27. Jaafaru MS, Karim NAA, Eliaser EM, Waziri PM,

Ahmed H, Barau MM, *et al*. Nontoxic glucomoringin-isothiocyanate (GMG-ITC) rich soluble extract induces apoptosis and inhibits proliferation of human prostate adenocarcinoma cells (PC-3). Nutrients. 2018;10(9).
28. Shu X sheng, Wang D dan, Zhao Y ying, Sun Y mei, Hu X peng, Huang X yan, *et al*. Extract from Moringa oleifera seeds suppresses the epithelial-mesenchymal transition-mediated metastasis of gastric cancer by targeting the metastatic suppressor NDRG1. J Funct Foods. 2018;50(September):93–103.
29. Asaduzzaman AKM, Hasan I, Chakrabortty A, Zaman S, Islam SS, Ahmed FRS, *et al*. Moringa oleifera seed lectin inhibits Ehrlich ascites carcinoma cell growth by inducing apoptosis through the regulation of Bak and NF-κB gene expression. Int J Biol Macromol. 2018;107:1936–44.
30. Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H, Islam M. Moringa oleifera as an anti-cancer agent against breast and colorectal cancer cell lines. PLoS One. 2015;10(8):1–14.
31. Rahaman MHA, Kadir NHA, Amin NM, Omar WBW. Cytotoxicity effect of water extracts of Moringa oleifera leaves and seeds against MCF-7 cells. Acta Hortic. 2017;1158(May):279–86.
32. Gaffar S, Apriani R, Herlina T. N-Hexane fraction of Moringa oleifera Lam. Leaves induces apoptosis and cell cycle arrest on T47D breast cancer cell line. J Pharm Pharmacogn Res. 2019;7(3):173–83.
33. Adebayo IA, Arsad H, Kamal NNSBNM, Samian MR. The hexane fraction of the Moringa oleifera Lam seed extract induces apoptosis, causes cell cycle arrest, and modulates expression of HSP60, NPM, PGK1, RCN1, and PDIA1 in MCF7 cells. South African J Bot. 2020;129:379–87.
34. Daulay A, Hasibuan P, Masfria. Effect of ethanolic extract and n-hexane fraction of Moringa oleifera L. against cell cycle and apoptosis MCF-7 cell lines. 2019;6(1):25–30.
35. Adebayo IA, Arsad H, Samian MR. Antiproliferative Effect on Breast Cancer (Mcf7) of Moringa Oleifera Seed Extracts. African J Tradit Complement Altern Med AJTCAM. 2017;14(2):282–7.
36. Ghosh N. Anticancer effect of Moringa oleifera leaf extract on human breast cancer cell. Fac Emgineering Technol Jadavpur Univ. 2013;1–57.
37. Hossain N, Mirghani MES, Raus RB. Optimization of Moringa oleifera Leaf Extraction and Investigation of Anti Breast Cancer Activity with the Leaf Extract. Eng Int. 2015;3(2):97.
38. Poobalan K, Lim V, Mohamed Kamal NNSN, Yusoff NA, Khor KZ, Samad NA. Effects of Ultrasound Assisted Sequential Extraction (UASE) of Moringa oleifera Leaves Extract on MCF 7 Human Breast Cell Line. Malaysian J Med Heal Sci. 2018;14:102–6.
39. Chen G., Yang M., Kuo P., Lin M. LM. Chemical constituents of Moringa oleifera and their cytotoxicity aganist doxorubicin-resistant human. Chem Nat Compd. 2014;50(1):154–6.
40. Taha M, Parveen B, Osman B, Abdoon IH, Mohamed MS, Osman WJA, *et al*. Original article In vitro profiling of plants used in Sudanese traditional medicine for antioxidant and anti-breast cancer activities. Ann Phytomedicine An Int J. 2019;8(1):119–26.
41. Guon TE, Chung HS. Moringa oleifera fruit induce apoptosis via reactive oxygen species-dependent activation of mitogen-activated protein kinases in human melanoma A2058 cells. Oncol Lett. 2017;14(2):1703–10.
42. Antonini E, Iori R, Ninfali P, Scarpa ES. A Combination of Moringin and Avenanthramide 2f Inhibits the Proliferation of Hep3B Liver Cancer Cells Inducing Intrinsic and Extrinsic Apoptosis. Nutr Cancer. 2018;70(7):1159–65.
43. Tumer TB, Rojas-Silva P, Poulev A, Raskin I, Waterman C. Direct and indirect antioxidant activity of polyphenol- and isothiocyanate-enriched fractions from moringa oleifera. J Agric Food Chem. 2015;63(5):1505–13.
44. Cuellar-Nuñez ML, Luzardo-Ocampo I, Campos-Vega R, Gallegos-Corona MA, González de Mejía E, Loarca-Piña G. Physicochemical and nutraceutical properties of moringa (Moringa oleifera) leaves and their effects in an in vivo AOM/DSS-induced colorectal carcinogenesis model. Food Res Int. 2018;105:159–68.
45. Kraiphet S, Butryee C, Rungsipipat A, Budda S, Rattanapinyopitak K, Tuntipopipat S. Apoptosis induced by Moringa oleifera Lam. pod in mouse colon carcinoma model. Comp Clin Path. 2018;27(1):21–30.
46. Sadek KM. Chemotherapeutic efficacy of an ethanolic Moringa oleifera leaf extract against chromium-induced testicular toxicity in rats. Andrologia. 2014;46(9):1047–54.
47. Michl C, Vivarelli F, Weigl J, De Nicola GR, Canistro D, Paolini M, *et al*. The chemopreventive phytochemical moringin isolated from Moringa oleifera seeds inhibits JAK/STAT signaling. PLoS One. 2016;11(6):1–20.
48. Tiloke C, Anand K, Gengan RM, Chuturgoon AA. Moringa oleifera and their phytonanoparticles: Potential antiproliferative agents against cancer. Biomed Pharmacother. 2018;108(September):457–66