# Therapeutic Potential of Flavonoids in Modulating Mitochondrial Activity in Liver Cancer: Insights from HepG2 Cell Studies

Ghazal Abdullahi Olawale[1], Nozlena Abdul Samad [1*], Muath H. S. Helal[2]

## Abstract

Background: Cancer, a leading cause of mortality globally, affects various organs, including the liver, with liver cancer contributing to approximately 830,000 deaths in 2020. In Malaysia, liver cancer is increasingly prevalent. The HepG2 cell line, derived from human hepatoma, is widely used for studying liver cancer and drug-induced liver injuries. Flavonoids, a group of polyphenolic compounds found in plants, have gained attention for their diverse pharmacological activities, including their potential to mitigate carcinogenesis and tumorigenesis in liver cancers. Methods: This review synthesizes findings from studies investigating the impact of flavonoids on liver cancer, focusing on the HepG2 cell line. We explore flavonoid subtypes and their therapeutic roles, including their antioxidant, anti-inflammatory, anti-angiogenic, and cytotoxic properties. Research on mitochondrial function in cancer cells and how flavonoids influence mitochondrial activity in the HepG2 cell line is also assessed. Results: Flavonoids demonstrated significant antioxidant properties by modulating reactive oxygen species (ROS) levels and maintaining mitochondrial homeostasis in HepG2 cells. Key flavonoids, including quercetin, naringenin, and kaempferol, exhibited strong anti-inflammatory and anti-angiogenic effects. Furthermore, cytotoxic flavonoids induced apoptosis in HepG2 cells through mitochondrial disruption and ROS generation, with certain compounds showing selective toxicity to cancer cells. Conclusion: Flavonoids exhibit promising therapeutic potential in liver cancer by targeting oxidative stress, inflammation, and angiogenesis while promoting apoptosis in HepG2 cells. Their ability to modulate mitochondrial function positions them as potent agents for further investigation in liver cancer therapy.

**Keywords:** Flavonoids, HepG2 cells, Mitochondria, Liver cancer, Antioxidants, Apoptosis, ROS.

***Significance*** | This review highlights the potential of flavonoids as therapeutic agents in liver cancer, focusing on HepG2 cell models.

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## Introduction

Cancer, the second leading cause of death worldwide, is a broad term for a group of diseases which can start from any part of the body when normal cells undergo a multi-stage process leading to uncontrollable abnormal growth, with the potential to spread to other body parts (metastasize). According to data from the World Health Organisation (WHO, 2020), cancer accounted for approximately 10 million deaths globally in 2020. Of the different types, liver cancer had the third highest toll, with approximately 830 thousand deaths in the same period. In Malaysia, liver cancer ranks as the eighth most common type of cancer, and it is even more prevalent in males, ranking as the fifth most common cancer. There has been an upward trend since 1990, with a significant increase in

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annual years of life lost (Mohamed et al., 2018). Cancer, a disease with a poor remission rate often involves metabolic abnormalities accompanied by comorbidities from other metabolic disorders.

The liver, considered the largest internal organ, and with a complex network of metabolic activities, is one of the most important organs in the human body. As the only organ with regenerative ability, the liver carries out a plethora of functions involved in digestion, detoxification, protein synthesis, etc. (Huang et al., 2022). Conversely, the diverse and versatile nature of the liver makes it very susceptible to cellular damage. Liver cancer typically arises from a combination of various, less ahrmful damages that impir the liver's functionality. Chronic viral hepatitis, non-alcoholic fatty liver disease (NAFLD), cirrhosis and inherited metabolic disorders are all factors that significantly predispose a person to liver cancer, with males estimated to possess a two- to three-times higher risk of the disease (Li et al., 2023). Researchers continue to conduct extensive research on liver cancer and other liver-related injuries.

The HepG2 cell line is an immortalised human hepatoma-derived cell line, most commonly used in studying drug metabolism and drug-induced liver injury, as well as hepatotoxicity (Goldring et al., 2020). Following its patenting in 1980 by the Wistar Institute, Arzumanian et al. (2021), argued that the HepG2 cell line was incorrectly labelled as a hepatocellular carcinoma (HCC), when, it was a hepatoblastoma (HB), citing the work of López-Terrada et al., (2009) on the nature of HepG2. History aside, the HepG2 cell line is a widely used model that has played crucial roles in gaining insights and understanding several hepatic processes and activities in liver cancers.

Flavonoids, secondary metabolites which are abundant in many foods and present since time immemorial, represent the largest and most diverse group of phytonutrients (Chand et al., 2023). A variety of plants and plant parts, such as fruits, grains, flowers, roots, and seeds, contain these polyphenolic compounds responsible for plant pigment formation (Liu et al., 2024). For many years, researchers have studied the complex interactions between dietary components and pharmacological activity, and their potential efficacy at the cellular level is an increasingly popular research area.

Chemically, flavonoids belong to the class of hydroxylated polyphenols and are composed of a 15-carbon skeleton (or C6-C3-C6 backbone), which consists of two phenyl rings and a heterocyclic ring (Chand et al., 2023). The phenylpropanoid pathway synthesises them, using phenylalanine as its basic building block, which comes from the shikimate pathway (Galatro et al., 2024). Beyond that core backbone, the stereochemical arrangement of, and on the rings (especially the central ring), all influence the grouping and subgrouping of flavonoids (Gupta et al., 2024). Naringenin, the most basic flavonoid, serves as an important intermediary for other flavonoids, making their pathway relatively conservative despite their numbers and diversity in nature (Tao et al., 2023). The flavonols, myricetin, kaempferol and quercetin are the most prevalent in plants, after naringenin.

The plant kingdom widely distributes several flavonoid subtypes, including flavanols, flavonols, flavones, flavanones, isoflavones, anthocyanins, chalcones, with their metabolic pathways widely studied through biochemical and molecular biological techniques (Chand et al., 2023). Currently, there exist over 10,000 identified and separated flavonoid compounds with many possessing pharmacological activities that are beneficial to man, owing to their ability to suppress inflammation, act as an antioxidant, aid bone formation, and inhibit cancer formation (Safe et al., 2021).

Flavonoids are responsible for diverse pharmacological activities, and garner widespread interest, especially due to their ability to scavenge free radicals. They possess anticancer, antimicrobial, antiviral, antiangiogenic, antiadipogenic, antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents (Ballard & Maróstica, 2019; Ullah et al., 2020; Rajan et al., 2022). They are also required for a wide range of pharmaceutical, nutraceutical, medical, cosmetic, and other uses (Gupta et al., 2024). In the context of liver health, particularly the HepG2 cell line, flavonoids have shown promise in modulating mitochondrial activity. Given the immensurable therapeutic opportunities that naturally occurring flavonoids present, we review the potential of some of these compounds to combat carcinogenesis and tumorigenesis in the HepG2 cell line – a widely studied and referenced hepatocellular model.

## Therapeutic applications of flavonoids

### 1. Flavonoids as potent antioxidants

The liver is an important site of oxidation prevention, consequentially, it is also one of the organs most seriously affected by oxidation due to the vigorousness of liver metabolism. The high oxygen consumption leads to the generation of excessive oxygen free radicals, known collectively as reactive oxygen species (ROS). Studies have shown that flavonoids work as antioxidants by keeping ROS levels in check by rebalancing of the enzymes that make ROS and the enzymes that neutralise them.

Reactive oxygen species (ROS) are highly reactive chemicals containing diatomic oxygen and readily react with biomolecules present in cells. Under physiological conditions, they are generated mainly as byproducts of the electron transport chain, but can also be generated from NADPH oxidases, nitric acid synthetases, etc, (Villalpando-Rodriguez & Gibson, 2021). They function as key components in many biochemical processes, such as protein phosphorylation, transcription factors initiation, immunity and apoptosis. Their importance in the body cannot be overemphasised. However, excesses of ROS can react with molecules such as proteins, nucleic acids and lipids, leading to oxidative stress, which in turn causes cell damage and death.

Various endogenous (detoxifying) compounds such as glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), or different exogenous antioxidants, such as flavonoids, ascorbic acids, and vitamin E, strongly regulate the levels of ROS in cells (Abdal Dayem et al., 2017). The most commercially used supplemented antioxidants are vitamins C and E, and their use is widespread.

A study by Li et al., (2023) suggested that flavonoids can help mitochondrial homeostasis and can increase the phosphorylation of nuclear factor erythroid 2-related factor 2 (Nrf2), an upstream regulator of antioxidant enzymes. This is true even though flavonoids can lower ROS production by chelating ions. The seven identified compounds in the study – hesperetin, baicalin, dihydromyricetin, puerarin, cyanidin-3-O-glucoside, quercetin and epigallocatechin-3-gallate – all possessed great antioxidant properties when studied *in-vitro* and/or *in-vivo*.

Jung et al., (2017) looked into how four flavonoids from Korean milk thistle (*Cirsium japonicum* var. *maackii*) protect HepG2 cells treated with tert-butyl hydroperoxide (t-BHP) from ROS generation. The flavonoids were luteolin (LT), luteolin 5-O-glucoside (LT5G), apigenin (AP), and apigenin 7-O-glucuronide (AP7G). hydroperoxide (t-BHP) from ROS generation. The flavonoids were luteolin (LT), luteolin 5-O-glucoside (LT5G), apigenin (AP), and apigenin 7-O-glucuronide (AP7G). They exposed HepG2 cells to t-BHP to initiate ROS generation, and then quantified it. Treating HepG2 cells with t-BHP up to about 100% significantly increased ROS generation. Pre-treating HepG2 cells with LT, LT5G, AP, or AP7G at varying concentrations significantly inhibited ROS generation and protected cells against ROS-induced oxidative stress in a concentration-dependent manner. LT, LT5G, and AP7G inhibited t-BHP-induced ROS generation significantly more than AP, which did not markedly inhibit ROS generation. The inhibitory effects of low-concentration luteolin t-BHP-induced ROS generation were found to be similar to those of the untreated control group, demonstrating that flavonoid compounds from *C. maackii* scavenge ROS induced by t-BHP in HepG2 cells.

**Flavonoids as effective anti-inflammatory agents**

Higher organisms develop inflammation as a complex defensive strategy to counteract the damaging effects of tissue injury, microbial infection, and other harmful conditions. This strategy aids in tissue repair and elimination of harmful stimuli. Despite its evident importance, long-term inflammation, particularly in certain cellular environments, can be extremely harmful, leading to cellular damage and diseases (Farzaei et al., 2019).

Flavonoids, a group of polyphenolic compounds, elicit suppressive actions on inflammation by inhibiting enzymes and cytokines involved in the inflammatory response. Previous literature on the effects of flavonoids on inflammatory response demonstrates that anti-inflammatory activity is mainly due to the inhibition of nuclear factor kappa B, NF-κB (González et al., 2011). They also modulate the activities of enzymes that metabolize arachidonic acid, modulate β-cell and antibody production, enhance natural killer cell cytotoxicity, inhibit Th17-dependent differentiation and NLRP3 (nucleotide-binding oligomerization domain-like-receptor family pyrin domain-containing 3) inflammation, and induce CD8+cells (Ballard & Maróstica, 2019; Ferraz et al., 2020; Li et al., 2023). Human intervention studies on the effects of flavonoids on immune responses present uncertainties, diminishing their efficacy and reliability in immune therapies (Safe et al., 2021).

**The role of flavonoids in angiogenesis**

Angiogenesis is the term that describes the process of natural formation of new blood and lymphatic vessels from a pre-existing vasculature. This process enables tumour cells to obtain sustenance in the form of nutrients and oxygen, as well as the capacity to eliminate metabolic waste, playing a key role in the process of tumorigenesis, because solid tumours require a blood supply if they are to grow in size beyond a few millimetres (Saman et al., 2020). As a result of this crucial role, researchers are now beginning to pay more attention to this process and its possible role in cancer management.

Plant polyphenols, such as flavonoids, inhibit angiogenesis by regulating multiple signalling pathways. Quercetin is a flavonol that has been extensively studied. It is known to help prevent colorectal cancer by inducing cell cycle arrest, increasing apoptosis, antioxidant replication, modulating oestrogen receptors, regulating signalling pathways, and inhibition of metastasis and angiogenesis (Darband et al., 2018). Al-Dabbagh et al., (2019) conducted a study on *Matricaria recutita L.* (chamomile) flower demonstrated a dose-dependent enhanced mortality of HepG2 cells in HCC. Its synthesised extract also showed a dose-dependent reduction of vascular endothelial growth factor, (VEGF) – which is crucial in angiogenesis – making it an efficient anticancer agent.

Another study by Aleksandar et al., (2019), on wild edible onions (*Allium flavum* and *Allium carinatum*) showed abundance of the flavonoids – rutin, quercetin 3-O-glucoside, and kaempferol-3-O-glucoside. The antioxidant potential of which are well researched, they are also effective anticancer agents, which when combined with Doxorubicin, upregulate angiogenic factors against human hepatoma (HepG2) and lung carcinoma (A549) cell lines. Kamble & Gacche (2019) used the chorioallantoic membrane (CAM) assay to test the antiangiogenic properties of MeOH extracts of *Cassia occidentalis, Callistemon viminalis, Cleome viscosa* and *Mimosa hamata*. Among the selected plant extracts, *C. viminalis* and *C. viscosa* leaves illustrated potent antiangiogenic activity by inhibiting the branching of blood vessels in the CAM model, whereas the remaining plant extracts also showed efficient inhibition. The antiangiogenic properties of plant extracts might be attributed to the high content of phenols and flavonoids present in them.

**Flavonoids as suitable and alternative cytotoxic agents**

Cytotoxicity in cancer refers to the ability of certain compounds or molecules to induce or potentiate apoptosis; and/or the ability to inhibit proliferation in cancer cells. Despite the presence of a sizeable number of cytotoxic agents approved for the management of different types of cancer, only a few inhibit oncogenesis, contrarily, many of them are toxic and have numerous adverse effects (Ullah et al., 2020). This highlights the need for safer alternatives that can effectively inhibit tumour growth while remaining safe (selective toxicity) to normal cells. Flavonoids have been extensively researched in this field with numerous isolated compounds or extracts yielding positive results.

A study by Li et al., (2017), on the anticancer activity of 5, 7-Dimethoxyflavone (DMF) in HepG2 cells showed that the compound was effective in causing cell death in the cells with an $IC_{50}$ value of 25μM. The compound elicited its cytotoxicity by inducing significant cell arrest at the sub-G1 phase in a dose-dependent manner, inducing apoptosis. The study showed that 5,7-DMF initiated a complex pathway involving an increase in ROS generation in and around the mitochondria, disrupting its membrane potential, leading to the activation of caspase cascade, then cell death.

Kuete et al., (2015), studied the cytotoxicity of two flavones (Artocarpesin and Cycloartocarpesin) and a chalcone (Isobavachalcone) against multi-factorial drug-resistant cells. The compounds were tested by using both the drug-sensitive cell lines and their corresponding resistant cell lines. They found all three compounds to show significant activity against both sets of cells with isobavachalcone having very promising values. Cycloartocarpesin and isobavachalcone were noted to cause G1 cell cycle arrest, causing apoptosis by activating Caspase 3/7, 8 and 9, and also via ROS generation. In all cases, the degree of resistance to the three tested flavonoids was found to be lower than that of doxorubicin, highlighting their potential to fight cancer multidrug resistance.

Another study by Kuete et al., (2014), on the activity of 4′-hydroxy-2′,6′-dimethoxychalcone against drug-sensitive and drug-resistant cell lines, showed the compound was highly cytotoxic, giving an $IC_{50}$ of <10μM against 5 of the 9 tested cell lines. The flavonoid was observed to cause an increase in sub-G0/G1 increase, causing apoptosis via disruption of mitochondrial membrane potential and ROS generation. Li et al., (2014), studied the effects of quercetin, isoquercetin and rutin on HepG2 cells. They observed that the compounds cause G2/M cell cycle arrest, inhibiting cell growth in a dose-dependent manner by inducing apoptosis via ROS generation.

**The role of mitochondria in normal cells and tumour cells**

A century ago, the mitochondria were not considered to play a significant role in cancer cells, mainly due to the observation of the German physiologist, Otto Heinrich Warburg. In 1924, Warburg found that cancer cells used glycolysis to yield adenosine triphosphate (ATP) and other essential molecules via the oxidation of glucose into lactic acid, despite possessing a fully functional oxygen-filled mitochondrial respiratory chain (Liu et al., 2021; Barba et al., 2024). This came to be known as the 'Warburg effect' or 'Aerobic glycolysis', and it brought the suggestion that oxidative phosphorylation was less important in tumour cells compared to normal cells, despite the tumour cells being able to fully utilise this pathway for ATP production.

At the time, Warburg also hypothesized that cancer formation was due to dysfunctional mitochondria resulting in a shift to aerobic glycolysis (Klein et al., 2020; Liu & Shi, 2020). Recent research into the mitochondria has, however, found this to not be true. Although aerobic glycolysis indeed occurs in tumour cells, many tumour cells also possess functional mitochondria.

The mitochondria, also known as the "powerhouse of the cell", are dynamic organelles of great importance as they possess the ability to rapidly adapt to stressful conditions, allowing cell survival, making them potential crucial targets for cancer therapy (Genovese et al., 2021). Mitochondria play key roles in ATP production, lipid metabolism, nucleic acid metabolism, mitochondrial fusion, mitochondrial fission, mitochondrial transfer, mitophagy, Ca2+ homeostasis, ROS generation, redox molecules generation, cell signalling and apoptosis (Liu & Shi, 2020; Genovese et al., 2021; Liu et al., 2023). Many pathologies, including cancer, are increasingly linked to alterations in mitochondrial DNA (mtDNA) and dysfunction. Numerous studies suggest that eliminating the mitochondrial DNAs in cancer cells can reduce the growth rates and tumorigenicity of those cells. The mitochondria influence cancer development and proliferation via some of the following:

**Mitochondria in bioenergetics**

Most of the ATP required by the body is generated in the mitochondria via a process called oxidative phosphorylation (OXPHOS), which is interconnected with the tricarboxylic acid cycle (TCA) and fatty acid oxidation (FAO) pathways, via Electron Transport Chain (ETC) – which involves the transport of electrons through a series of complexes in the inner membrane. Numerous proteins and complexes in this OXPHOS pathway have been identified as cancer targets (Liu et al., 2023). Heterogeneity of tumours allows for the exhibition of differing metabolic phenotypes, with proliferating tumour cells preferring a shift towards increased glycolytic metabolism from OXPHOS in the presence of $O_2$, while slow-cycling ones may opt for mitochondrial respiration as a primary source of energy (Liu et al., 2023).

In comparison, despite having less ATP production efficiency, aerobic glycolysis is more beneficial to rapidly proliferating cells as it boosts the formation of other required biomass precursors (Klein et al., 2020). Contrarily, the OXPHOS pathway produces significant ATP for cancer cells, suggesting the preservation of some mitochondrial function in cancer

In areas of low glucose concentration, cells utilise other carbon sources, such as lactate, for efficient mitochondrial ATP production, with the NADH and FADH2 generated from glycolysis and TCA cycle entering the matrix in mitochondria (Liu et al., 2023). This allows for the synthesis of broader substrates, which in turn enables the conduction of extensive and high plastic metabolic rewiring by the tumour cells, upping their survival chances in otherwise harsh nutrient conditions (Liu & Shi, 2020). Tumorigenic potential of numerous cancer types is bolstered by depending more on OXPHOS rather than glycolysis for ATP production. Cancer cells generate resistance by upregulating OXPHOS and TCA cycle to acquire more ATP than their surrounding normal cells, thus creating the possibility of potent OXPHOS inhibitors to induce lethargic mitochondrial stress, ranging from mild to severe.

**Mitochondria in biosynthesis and $Ca^{2+}$ signalling**

As a central metabolic organelle, mitochondria provide the intermediates needed for biomass synthesis, including fatty acids, amino acids, and nucleotides, which serve as building blocks for cancer cell growth (Liu & Shi, 2020). The mitochondria synthesises mitochondrial membrane lipids, while the endoplasmic reticulum (ER) synthesises other lipids. Intra-mitochondrial lipid homeostasis and transport inside the cell are critical to mitochondrial respiratory function. In addition, lipid peroxidation (LPO) – one of the markers for oxidative stress – may occur, initiating secondary cellular responses, and damaging DNA, proteins and enzyme activity. It activates signalling pathways, initiating cell death.

Purine and pyrimidine nucleotides are basic building blocks in the synthesis of RNA and DNA, which are in turn, essential processes in cell division and proliferation. A high ATP/ADP ratio is necessary to maintain energy precursors for RNA synthesis, and lower than required levels of ATP may result in cessation of corresponding cell functions or even in necrotic cell death. This implies that cell proliferation via nucleic acid production involves adjustments to both energy metabolism and the nucleotide biosynthetic pathways, with most cells synthesising nucleic acids anew, mainly from glucose, glutamine and CO2. In the TCA cycle, aspartate is the intermediate for synthesising nucleotide bases (Liu et al., 2023).

Cell signalling pathways are interconnected, with a wide range of functions, and mutations in proteins involved in cell signalling pathways such as MEK, extracellular signal-regulated kinase (ERK), H-Ras, Raf, NRAS, and BRAF are key factors in the development of cancer, with their deregulation frequently observed in many tumour cases (Devi et al., 2015). Regulation of cell Ca2+ homeostasis involves the mitochondria, making it essential for a myriad of cell functions (Genovese et al., 2021). Mitochondrial Ca2+ signalling intricately links cell growth and metabolism by activating multiple components of the TCA cycle that feed the ETC thereby increasing ATP production (Missiroli et al., 2020).

Increased mitochondrial Ca2+ may trigger cell death by necrosis or cell death related to the continuous opening of mitochondrial permeability transition pore (mPTP) and the release of Cytochrome-C, thus representing a key program for cell fate decisions. Interestingly, mPTP is often found inactivated in most cancer cells, making it a likely survival mechanism in unsuitable conditions (Farzaei et al., 2019; Liu & Shi, 2020). Some cells have developed mitochondrial Ca2+ influx and efflux systems, which become extremely helpful in tumorigenesis due to its nuanced role in regulating the survival/death fate of a cell.

However, chemotherapeutic agents may trigger a pharmacokinetic interaction which either directly or indirectly (Ca2+ uptake) affects mitochondrial Ca2+ homeostasis (Genovese et al., 2021). These agents reduce the mitochondrial Ca2+ accumulation , preventing cancer cell apoptosis; contrastingly, they also elevate the mitochondrial calcium uniporter, MCU-dependent mitochondrial Ca2+, which has been associated with invasion, metastasis and poor prognosis due to increase in NADH/NAD+ ratio via TCA cycle stimulation (Missiroli et al., 2020).

**2. The relationship between the mitochondria and the tumour microenvironment; Mitochondrial hypoxia- and ROS-induced apoptosis**

Comprising of tumour cells, immune cells and the surrounding environment of tumours, the heterogeneous TME plays pivotal roles in tumour imitation, progression and metastasis; requiring varying oxygen supply, which in turn leads to the diversity of mitochondrial distribution (Liu et al., 2023). In 1954, Argentinian physiologist, Rebecca Gerschman, theorised the toxic effects of oxygen due to partially reduced forms known as free radicals. She noted that compounds were generated as a byproduct of metabolic processes, and include the superoxide anion ($O_2^-$), singlet oxygen ($1O_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (-OH) (Gerschman et al., 1954).

They are believed to act as second messengers in cell signalling, possessing dual cellular roles, and participating in intracellular signalling (including potentially oncogenic signalling pathways) and innate immune responses at low doses; while high levels may lead to increased mtDNA mutagenicity, owing to the propinquity of mitochondrial ROS and mtDNA and the diminished "proofreading" capacity of mtDNA (Klein et al., 2020; Missiroli et al., 2020; Genovese et al., 2021). This causes oxidative stress, damaging organelles and biomolecules (DNA, proteins, and lipids), leading to cancer development through cellular inflammatory response. To counterpoise ROS production, enzymatic (such as superoxide dismutase, catalase, glutathione peroxidases, and thioredoxin), as well as nonenzymatic antioxidants are adopted by the cells to collectively exert antioxidant actions (Genovese et al., 2021).

Elevated ROS levels are usually due to suppression of the antioxidant system, and mitochondrial ROS (mROS) generation

(Genovese et al., 2021). Cancer cells produce more mROS than differentiated cells, primarily at the inner mitochondrial membrane during OXPHOS, where approximately 12% of molecular oxygen ($O_2$) is converted to anion superoxide ($O_2^-$) (Klein et al., 2020; Missiroli et al., 2020; Liu et al., 2023). At physiological levels, intracellular signalling and cancer cell growth are carried out by ROS, contrastingly excess ROS cause nuclear or mitochondrial DNA (mtDNA) mutations to accumulate, by directly affecting multiple biological processes. This eventually activates the DNA effector or cell death pathway, causing autoimmunity or apoptosis (Liu & Shi, 2020; Missiroli et al., 2020).

On the other hand, tumour cells express high levels of antioxidant proteins to avoid ROS-induced cell death by preventing accumulation (Missiroli et al., 2020). mtDNA is closely related to the electron transfer chain and lacks adequate repair capacity, making it a main target of ROS (Liu et al., 2023). The effect of hypoxia on cellular signalling pathways also plays a key role in cellular survival, proliferation, and metastasis, with aberrant signalling pathways allowing cancer cells to adapt of hypoxic environment (Klein et al., 2020). Hypoxia-mediated mROS generation activates hypoxia-inducible factor-1 (HIF-1) through overexpression of glycolytic enzymes, leading to the metabolic switch from OXPHOS to glycolysis, facilitating tumorigenesis and metastasis (Missiroli et al., 2020).

Mitochondria play a critical role in cell apoptosis (programmed death), mediating the intrinsic apoptosis program characterized by Cytochrome-C release (Ma et al., 2020). The Bcl-2 family proteins regulate this release and govern the mitochondrial outer membrane permeabilization (MOMP) (Liu & Shi, 2020). However, apoptosis is generally evaded by cancer cells via mechanisms that contribute to overexpression of antiapoptotic proteins, underexpression of pro-apoptotic proteins, reduced expression of caspase, increased expression of inhibitor of apoptosis proteins (IAPs), and defects in tumour suppressor proteins. Hence, the main target of cancer treatment is the restoration of the apoptosis machinery and the selective elimination of cancer cells (Devi et al., 2015).

Bcl-2 family proteins either function as preventing apoptosis (prosurvival) or inducing apoptosis (proapoptotic). It is now known that these Bcl-2 proteins possess dysfunctional regulation in many cancers with overexpression of prosurvival members or underexpression of proapoptotic members, aiding survival of cancer cells (Liu & Shi, 2020).

## 3. Mitochondrial dynamics, its role in Cancer Stem Cells (CSC)

The dynamics surrounding the frequent occurrence of both fission and fusion within the mitochondria, are crucial for maintaining morphology, regulating cell cycle and function. In the G1 and G2 phases, mitochondria form an interconnected network but become fragmented during the S and M phases (Ma et al., 2020). Impaired mitochondrial dynamics have been associated with the onset, progression and metastasis of several cancer types, CSC survival, and chemoresistance, thus suggesting that targeting mitochondrial dynamics is a potential therapeutic strategy for fighting cancer (Genovese et al., 2021).

Mitochondrial fusion involves the merging of two separate and distinct mitochondria into one, while mitochondrial fission is characterised by the splitting of an individual mitochondrion into two separate mitochondria (Genovese et al., 2021). In most cases, mitochondrial fission is involved in cancer development by facilitating the proliferation, metastasis and drug resistance of cancer cells, however, in some cancers, mitochondrial fusion has also been found to promote malignant phenotypes of cancer cells (Ma et al., 2020). Liu et al., (2023) states that high levels of mitochondrial fission activity are often linked to cancer cells that are highly proliferating and invasive, with adequate self-renewal and resistance to differentiation.

A study by Huang et al., (2016), showed elevated ROS production facilitates amplified mitochondrial fission, elevating HCC cells survival *in vitro* and *in vivo*. However, another study by Li et al., (2020), showed that mitochondrial fusion also aided liver tumour cell growth. Additionally, mitochondrial quality control and mitophagy facilitate proper mitochondrial function, with evidence supporting the coordination of mitochondrial fission with mitophagy, indicating a synergistic mitochondrial degradation pathway (Genovese et al., 2021). Mitophagy refers to the selective degeneration of damaged mitochondria via the formation of an autophagosome, which isolates the organelle, allowing its autophagosomal degradation following lysosomal fusion (Genovese et al., 2021).

Cancer stem cells (CSC) or tumour-initiating cells, refers to a subgroup of metabolically distinct and self-renewing tumour cells possessing high stemness and tumorigenicity responsible for tumour initiation, maintenance, growth, metastasis, recurrence and drug resistance (Liu & Shi, 2020; Liu et al., 2023). Despite relying highly on OXPHOS for energy, they also possess a more powerful antioxidant defence system (than bulk cancer cells) which can counteract and scavenge ROS to keep their ROS level low, maintaining stemness and tumorigenicity (Liu et al., 2023). They also possess high mitochondrial membrane potential, owing to mitochondrial fission and fusion.

Regulation of mitochondrial activities (mitochondrial reprogramming) is a crucial feature of CSCs, thus inhibiting fission and translation in the mitochondria or targeting the OXPHOS (or related key genes) pathway are potent therapeutic strategies against CSCs, making the mitochondria a therapeutic target for eradicating CSC growth and proliferation (Liu et al., 2023). Chemoresistance, which is a common cancer cell survival mechanism upon apoptotic stimulation is unavoidably connected to mitochondrion-related pathways (Genovese et al., 2021). Although mitochondrial fission physiologically represents the opposite process of mitochondrial

fusion, increasing evidence suggests mitochondrial fission similarly plays a key role in chemoresistance (Genovese et al., 2021).

**Flavonoids in halting/reversing mitochondrial damage or dysfunction**

A study by Rajan et al., (2022), on the effects of five citrus flavonoids (hesperidin, narirutin, nobiletin, sinensetin, and tangeretin) on AMPK activation in palmitate-treated HepG2 cells found the flavonoids significantly reduced intracellular lipid accumulation and enhanced glucose uptake in an insulin-independent manner. The activation of AMPK is involved in regulating liver lipogenesis, lipid oxidation, and cholesterol synthesis via protein phosphorylation or differential gene expression. The flavonoids were found to inhibit the expression of crucial components involved in these processes**.**

Another study by Ye et al., (2020), found pinocembrin, galangin and chrysin to be effective in alleviating ROS production, and also suppress the activation (phosphorylation) of extracellular signal-related kinases, ERK1/2. They also inhibited the nuclear translocation of the aryl hydrocarbon receptor (AHR), decreased CYP1A1 expression in ethanol-challenged HepG2 cells, showing their utility in attenuating ROS production and lipid accumulation.

Teekaraman et al., (2019), studied the apoptotic effects of quercetin in the PA-1 cell line. The study found quercetin to be highly effective against the cells with an $IC_{50}$ value of 75 µM, displaying DNA fragmentation after 24h of treatment. The flavonoid also decreased the protein and mRNA levels of the Bcl-2 family (anti-apoptotic) proteins present in the cells, causing apoptosis, by inducing the release of Cytochrome-C into the cytosol, triggering the activation of Caspase 9, and then, Caspase 3.

Feng et al. (2016) studied water-soluble total flavonoids (WSTF) isolated from *Isodon lophanthoides var. gerardianus* (Benth.) H. Hara demonstrating their cytotoxicity on HepG2 cells. Its suggested mode of action was related to inducing apoptosis and initiating cell cycle arrest at the G0/G1 phase. It was also noted to increase intracellular ROS and induce the release of cytochrome C. Vixetin and Rutin – both components of WSTF – act by regulating crucial signalling pathways, which inhibit growth, causing apoptosis. Autophagy was also suspected to play a role in the cytotoxicity of WTSF.

A review of the abilities of myricetin by Devi et al., (2015), on its ability to target various signalling pathways in cancer showed its potential to inhibit and/or reverse mitochondrial damage. The authors found it to be effective in acting on key signalling proteins involved in normal physiologic cellular processes, malignant transformation, tumour progression, tissue invasion and metastasis. Examples of such proteins included JAK1–STAT3 (Janus kinase–signal transducers and activators of transcription 3), MEK (mitogen-activated protein kinase, MAPK), Akt (Protein Kinase B) and Fyn protein. In HepG2 cells, myricetin was found to significantly cause nuclear fragments and condensed chromatin, leading to a cascade of pro-apoptotic events ending with the death of the cells, making it an effective chemotherapeutic agent against hepatocellular carcinoma.

**Synergistic effects of flavonoids and other bioactive compounds**

Although extensive research has been carried out into the biological activities of flavonoids, lesser attention has been paid to the possible synergistic effects of these biomolecules. Interactions between compounds are generally classified as synergistic, additive or antagonistic. Of the 3 types, synergy is the most desired in the field of oncology. This is due to the development of resistance to available drugs and the difficulty associated with new drug discoveries to combat drug-resistant and recalcitrant tumours.

Synergy involves combining two or more compounds to yield a greater effect than the sum of the individual effects of the compounds. Synergy may also occur via the regulation of the absorption, distribution, metabolism, and excretion of one compound by another compound, enhancing its the therapeutic effect or when all compounds involved are inactive on their own but become active when combined (Vaou et al., 2022).

The HepG2 cell line is without a doubt, highly researched, while research into potential cytotoxic flavonoids has been extensive over the years. There is, however, no known research by the authors in which the synergistic effects of flavonoids and approved anticancer agents have been carried out. Flavonoids themselves are usually in multitudes in their bio-source, and many research uses extracts of plant parts to test for activity i.e. not necessarily isolating individual flavonoid compounds. This may be proof that flavonoids may possess synergistic effects with one another, to elicit therapeutic activity. However, this is mere speculation and more research still needs to be done to determine the various interactions that may occur amongst different flavonoid compounds – obtained from extracts of the same source.

Despite this, more research is being carried out in other cell lines, trying to achieve a synergistic effect – by combining flavonoids with conventional anticancer drugs – to combat the current challenges in the management of cancers. A systematic review by Asnaashari et al. (2023), on the synergistic effects of numerous flavonoids with Paclitaxel on numerous cancer types. The study found out that many – already identified and isolated – flavonoids were able to potentiate therapy, elicit selective cytotoxicity, reduce adverse reactions, decrease drug resistance and prevent remission in cancer patients. This shows the potential of flavonoids to serve as beneficial adjuvants in cancer management.

**Therapeutic Potential and Clinical Implications**

The review presents a detailed investigation into the therapeutic potential of naturally occurring flavonoids, with a focus on their impact on the mitochondrial activity of HepG2 cells, a widely

researched model for liver cancer research. The study highlights the diverse role of flavonoids in various pathophysiologic conditions, illustrating their significance in modulating mitochondrial function and combating oxidative stress. The ability of these biologically abundant compounds to regulate the generation and/or action of reactive oxygen species (ROS), enhance mitochondrial homeostasis and target different tumour hallmarks is crucial to their anticancer properties.

The clinical implications of flavonoids in cancer treatment are vast. Flavonoids could offer a more natural and less toxic alternative to conventional chemotherapy drugs. Their ability to target multiple cancer-related pathways could lead to more effective treatments with fewer side effects. Additionally, the potential for synergistic combination therapies could enhance the efficacy of existing therapies and possibly reduce drug resistance.

## Conclusion

In conclusion, flavonoids hold promise as potential therapeutic agents in the treatment of liver cancer, as their ability to target multiple pathways involved in tumorigenesis posits them as valuable compounds in the development of anticancer strategies. However, there is a need for continued exploration of flavonoids, especially for their role in maintaining mitochondrial function and integrity and mitigating liver cancer progression.

## Author contributions

G.A.O. contributed to the design of the manuscript, conducted the literature review, and drafted the manuscript. N.A.S. supervised the design and structure of the manuscript and gave final approval of the version to be published. M.H.S.H. assisted in the analysis of the literature. All authors read and approved the final manuscript.

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## Competing financial interests

The authors have no conflict of interest.

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