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REVIEW OF THE SIGNIFICANCE OF STATINS AND THE THERAPEUTIC BENEFITS OF SILYBIN MARIANUM IN THE TREATMENT OF LIVER CANCER

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The therapeutic benefits of milk thistle, attributed largely to silymarin, a flavonolignan derived from the milk thistle Silybum marianum (L.), have long been used in traditional medicine. Silymarin has been shown to have a variety of pharmacological effects, including hepatoprotective, antioxidant, anti-inflammatory, antitumor, and cardioprotective activity. The derivatives and secondary metabolites of this plant have a variety of anti-inflammatory and anticarcinogenic characteristics. SC-CO2 was a new and environmentally friendly technology used to extract these powerful metabolites from silybin of Albanian origin. The extract included substantial amounts of linoleic and oleic acids, with 32% and 26.4%, respectively, detected in the extract. These significant percentages are attributed to the multiple benefits of milk thistle. This article addresses the effects of medical treatments such as statins along with the beneficial effect of milk thistle on liver cancer.

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INTRODUCTION

Cholesterol is a waxy substance that naturally occurs in cells and plays a vital role in the body's digestion, hormone production, and vitamin absorption, including vitamin D. However, everyone's cholesterol levels vary, and high levels of blood cholesterol can increase the risk of cardiovascular disease.

In 1987, the FDA authorized the use of statins as the primary tool for reducing cholesterol. Statins work by inhibiting the active site of HMG-CoA reductase, which is the first and most important rate-limiting enzyme in the mevalonate pathway.

This reduces hepatic cholesterol synthesis, leading to an increase in microsomal HMG-CoA reductase production and cell surface LDL receptor expression. Studies have shown that statins prevent the production of certain enzymes needed to make low-density lipoprotein (LDL), commonly referred to as "bad" cholesterol, and help the body process excessive cholesterol that may have accumulated over time. While the exact mechanism of statins is complex, there is plenty of evidence to explain why and how they lower LDL cholesterol.

The most popular statins include atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin. However, statins have a dose-dependent effect, and statin therapy is not always well tolerated, despite its widespread use, mainly because

of the side effects that are known to be possible (hepatotoxicity and myopathy). Despite that, recently, it has been noticed that the use of statins can be used as a *treatment for cancer*.

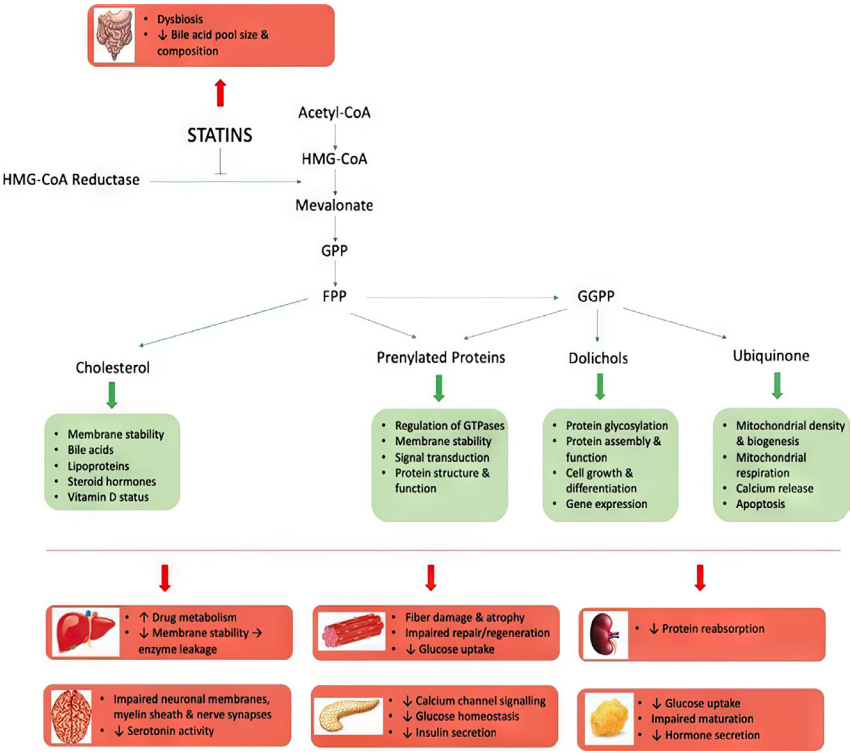


Figure 1. A possible mechanism for statin toxicity. FPP stands for farnesyl pyrophosphate; GGPP stands for geranylgeranyl pyrophosphate; GPP stands for geranyl pyrophosphate; and HMG-CoA reductase stands for hydroxymethylglutaryl-coenzyme A reductase [1]

The possibility of statins in cancer treatments was examined in many studies. It covered the current state of knowledge regarding the mechanisms underlying the anticancer abilities of statins and their impact on various cancers. The genesis of cancer metastases can be divided into different phases conceptually [2]. During the first phase, a subset of epithelial, E-cadherin (E-cad) expressing primary tumour *cells* undergoes partial or complete epithelial-to-mesenchymal transition (EMT), which includes E-cad expression downregulation [3]. This allows them to detach from the main tumour (as single cells or clusters of cells) [4], move through a barrier matrix and enter the circulation. During this pathway, some of these circulating cells survive and colonize distant organs by partially reverting to an epithelial phenotype via the *mesenchymal-epithelial reversion transition (MErT)* [5]. These cells frequently enter a state of dormancy [6; 7], during which the cells maintain very close contact with the untransformed environment [5]. This phase is followed by a growth phase in which the initially latent micrometastases grow in size, requiring at least a partial EMT [2].

A team of Johns Hopkins researchers conducted a trial to see which medications had the best "kill rate" of cells genetically modified to contain a mutation in a cancer gene called PTEN, a tumour suppressor enzyme that helps regulate cell division by preventing cells from growing and dividing (proliferating) too quickly or in an uncontrolled manner. The majority of the

2,500 medicines examined had no effect or killed both regular and genetically modified cells [8]. Certain molecular types of cancer may be more sensitive to statin therapy than many others. The question of whether statins have clinical anticancer effects is still being investigated. Statins appear to improve the efficacy and address the deficiencies of conventional cancer treatments, implying that statins should be examined in the context of cancer combination therapy.

Many researchers have investigated the role of statins on cancer cell growth and the current study found that statin use was a protective factor for the prognosis of rectal colon cancer [9]. Statins have antiproliferative effects in several forms of cancer [12] by reducing the synthesis of cholesterol and its metabolites [13, 14].

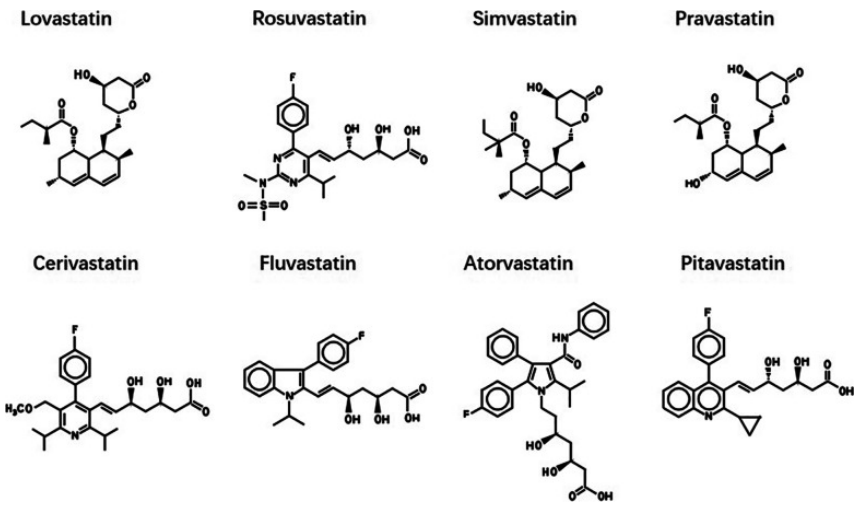


Figure 2. Chemical structures of some statins [15]

Treating liver cancer is complicated. That is where statin drugs may be useful. Certain statins appear to be more effective than others at avoiding liver cancer. Lipophilic statins (those that dissolve more easily in lipids such as oils and fats) may be more effective than hydrophilic statins in preventing liver cancer (those that dissolve more readily in water). This hypothesized difference is confirmed by studies that lipophilic statins can enter the damaged liver cells more easily, penetrating easily through cell walls consisting primarily of lipids. Lipophilic statins may be more effective at interfering with cancer formation once inside cells [16].

Lipophilic statins were linked to significantly lower HCC (hepatocellular carcinoma) incidence and mortality in a national viral hepatitis cohort. There was no evidence linking hydrophilic statins to a lower incidence of HCC (hepatocellular carcinoma). To assess whether lipophilic statin medication is practical for HCC prevention, more research is required [17]. Based on information gathered from the Swedish national registry, (a test performed primarily on people from Sweden, excluding persons with liver illness from causes other than viral hepatitis), their findings show that people who took a lipophilic statin (in this case atorvastatin or simvastatin) had a much lower probability of acquiring liver cancer than those who did not (3.3% versus 8.1%). As opposed to those who did take hydrophilic statins(such as rosuvastatin or pravastatin), the study did not discover a statistically significant reduction in

the incidence of liver cancer. When compared to not taking any statins at all, using either kind of statin was linked to a lower risk of liver cancer.

Two independent reviewers conducted a thorough literature search of PubMed, EMBASE, Google Scholar, Web of Science, and Scopus for publications published between January 1, 1990, and September 1, 2019 [18]. The results showed that statin use was linked to a lower risk of developing HCC compared to non-users. And when compared to non-users, the group that used statins, but also had concomitant diseases such as diabetes, cirrhosis of the liver or was also treated with antiviral drugs, it was found that the rate of reduction of HCC was also significantly higher.

MECHANISM

The mevalonate (MP) pathway is also known as the *isoprenoid pathway* or 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) [10]. This pathway is very important for the human body and is an anabolic mechanism that provides metabolites for many cellular functions in eukaryotes, archaea, and some bacteria. HMGCR converts acetoacetyl-CoA to mevalonate, which is then transformed into sterol isoprenoids such as cholesterol, which is an essential precursor of bile acids, lipoproteins, and steroid hormones, as well as several hydrophobic compounds such as dolichol, heme -A, isopentenyl tRNA, and ubiquinone. *Intermediates of this network play critical roles in the post-translational modification of a broad range of proteins involved in intercellular and intracellular signalling*. Statins may have non-lipid-related pleiotropic effects in addition to lowering LDL-c and cardiovascular morbidity and mortality. These include improved endothelial function, plaque stabilization, anti-inflammatory, immunomodulatory, and antithrombotic effects, effects on bone metabolism, and a lower risk of dementia.

Statins compete with HMG-CoA for binding to the active site of HMGCR, reducing mevalonate synthesis. As a result, statins deplete intracellular cholesterol, triggering a homeostatic feedback mechanism governed by the transcription

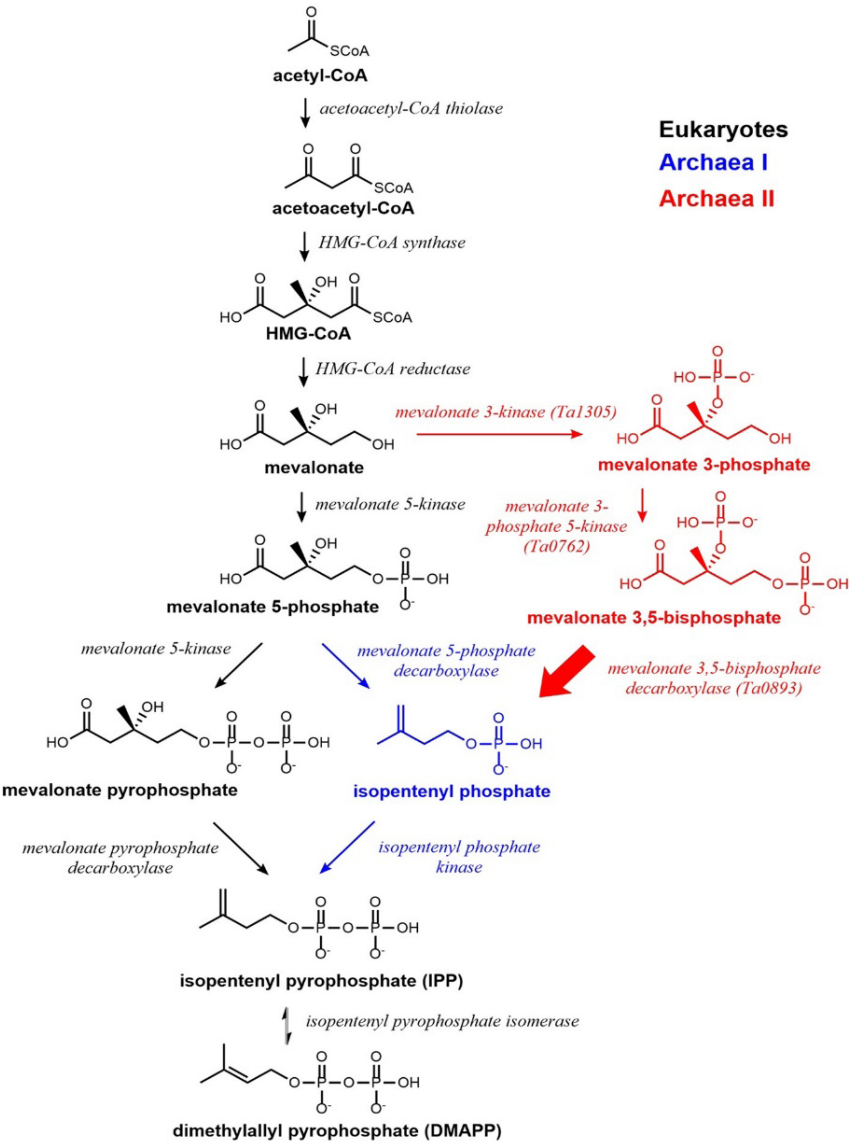


Figure 3. The Mevalonate pathway [55]

factor family *sterol regulatory element-binding protein* (SREBP) [19]. SREBP activation increases the expression of the mevalonate pathway and sterol metabolism genes, including HMGCR and the low-density lipoprotein (LDL) receptor (LDLR). Increased LDLR membrane expression leads to increased LDL cholesterol (LDL–C) uptake from the bloodstream, lowering serum cholesterol levels. As a result, statins are frequently prescribed to reduce the risk of cardiovascular disease or to improve survival in patients who already have it.

A review done for the interpretation of the role of lipids in cancer cell metabolism showed that during cancer progression, reprogramming lipid metabolism is critical for providing energy, macromolecules for membrane synthesis, and lipid-mediated signalling [20] highlights more recently recognized roles for lipids, particularly cholesterol and its derivatives, in cancer cell metabolism within intrinsically harsh tumour microenvironments.

How everything relates

Cholesterol is a necessary component of cell membranes, the structures that surround every cell in the human body. Cholesterol serves also as a precursor for downstream products such as steroid hormones and oxysterols: steroid hormones drive the initiation and progression of cancers such as breast and prostate carcinomas [21] increased oxysterol

production can activate the liver X receptors (LXRs), [22] which has been reported to affect the proliferation and survival of different cancer cell types that show altered metabolic pathways and cholesterol accumulation [23; 24].

As a result, cancer cells require cholesterol for growth and survival and inhibiting intracellular cholesterol biosynthesis is a promising anti-cancer treatment. The cellular cholesterol level reflects the dynamic balance of biosynthesis, uptake, export, and esterification – a process by which cholesterol is converted to neutral cholesteryl esters for storage in lipid droplets or secretion as lipoprotein constituents. In cancer cells, these cells are frequently mutated or dysregulated. A higher intracellular cholesterol level due to increased uptake by LDLRs *(low-density lipoprotein receptor),* decreased efflux by ABC transporters *(ATP-binding cassette),* and upregulation of de novo synthesis can sustain the metabolic need for cancer cell proliferation [25–26], and cholesterol accumulation has been described in many types of tumours [26; 27; 28; 29].

Cells typically obtain cholesterol through a variety of mechanisms, including direct synthesis via SREBP (*Sterol regulatory-element binding proteins*) transcriptional activity, which promotes the transcription of enzymes involved in cholesterol and fatty acid biosynthesis [3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) reductase] [30; 31]. *The recent use of HMG-CoA*

inhibitors (Statins) to block the mevalonate pathway and cholesterol de novo biosynthesis showed promising results in this regard [32]. However, cancer cells frequently gain a selective proliferative advantage by increasing LDLR-mediated uptake of exogenous cholesterol [33], making these therapies ineffective.

Glioblastoma multiforme (GBM), is one of the most well-known cases of cancer addiction on cholesterol. LXR agonists induced the low-density lipoprotein receptor degradation and increased apoptosis in glioblastoma cells expressing mutant epidermal growth factor receptor (EGFR), where tumour growth and survival are strongly dependent on SREBP-1-mediated lipogenesis since [33], SREBP-1-regulated lipogenesis in cancers including colorectal, prostate, breast, and hepatocellular cancer [34].

Furthermore, LXR activation increases cellular cholesterol efflux by ABCA1, lowering its levels and causing severe GBM cell death. As a result, LXR agonists (e.g., LXR-623) extended the survival of GBM-bearing mice models, indicating that targeting cholesterol metabolism may be a promising strategy in the treatment of this cancer [35; 36]. SREBP-1-mediated lipogenesis is essential for survival [33]. Furthermore, LXR activation increases cellular cholesterol efflux by ABCA1, lowering its levels and causing severe GBM cell death. As a result, LXR agonists (for example, LXR-623) extended the survival of GBM-bearing mice models, indicating that targeting

cholesterol metabolism may be a promising strategy in the treatment of this cancer [35; 36].

Activation of LXRs in various cancer models, including prostatic carcinoma, colon, mammary, and skin cancer, has been shown to have anti-proliferative effects due to the destruction of growth signalling pathways and the activation of pro-apoptotic signals [29]. LXRs have been shown to reduce the expression/activity of cell-cycle regulators, such as S-phase Kinase-associated protein (SPK2) in cancer cell lines [37], while also inducing the expression of cell-cycle inhibitors, such as p21 and p27 (cyclin-dependent kinase inhibitors) in prostate and ovarian cancer cells, with a concomitant decrease in phospho-RB protein levels [38; 39]. Furthermore, activation of LXRs in mouse models slowed the progression of androgen-dependent tumours toward androgen independence [38; 40].

In addition to these direct effects on cancer cell metabolism and survival, experimental evidence has recently revealed the importance of LXRs in anti-tumour immune responses. The role of LXR activation in this context is still in discussion. The activity of two transcription factors, the liver X receptors (LXR1 and LXR2) and the sterol regulatory element binding proteins (SREBP1 and SREBP2), is largely responsible for maintaining cellular cholesterol levels (41). Several tumours can produce oxysterols, which play an important role in cholesterol homeostasis by activating LXRs

[42; 43], and many of these metabolites can inhibit cancer cell proliferation [44]. Indeed, research has shown that oxysterols play a crucial part in regulating the way how tumour-infiltrating DCs produce the chemokine (C–C motif) receptor 7 (CCR7) [45].

It was discovered that LXR-dependent down-regulation of the chemokine (C–C motif) receptor 7 (CCR7) by tumour-derived oxysterols. CCR7, a member of the GPCR family that was first found as a gene up-regulated during an EBV infection, is essential for causing DC to migrate to draining lymph nodes. As a result, inhibiting CCR7 would effectively cause DCs to become trapped in the tumour, obstructing antigen presentation to antitumor T cells and host antitumor immunity. The activation of LXR in DCs was necessary for the oxysterol-mediated down-regulation of CCR7. Given the most recent evidence that oxysterols serve as chemoattractants, it would be interesting to investigate the oxysterols found in tumour supernatants for their capacity to chemotactic DCs without the involvement of LXR signalling [46]. Inhibiting the oxysterol-CXCR2 axis prevents tumour growth and extends the overall survival of mice with tumours, as shown in the study. These findings reveal a previously unknown protumor activity of the oxysterol-CXCR2 axis and suggest a potential target for cancer treatment [47]. Circulating and tumour-derived oxysterols have also been shown to recruit pro-tumor neutrophils,

increase neoangiogenesis, and suppress immune function in a CXCR2-dependent and LXR-independent manner [47; 48]. This demonstrates the ability of certain oxysterols to regulate a wide range of pro-tumour activities, depending on the LXR isoform expressed by the tumour cell's origin tissue and the surrounding microenvironment. Furthermore, 27-hydroxycholesterol has been shown to act as an estrogen receptor agonist in breast cancer, promoting tumour growth and metastasis [49].

LXRs, on the other hand, have been shown to control cancer cell growth by inducing LXR-dependent pyroptosis of cancer cells, and activation of LXR in macrophages has been shown to promote the phagocytosis of dying cancer cells [50]. ApoE induction, a transcriptional target of LXR, can cause MDSC depletion by activating the low-density lipoprotein receptor-related 8 (LRP8) receptor on these cells and potentiating cytotoxic lymphocyte activation. LXR activation combined with PD-1 inhibition improved the efficacy of cancer patients' cytotoxic T lymphocyte (CTL) and natural killer (NK) cells in these settings [51]. In a different scenario, activation of LXRs could increase the expression of MHC class I polypeptide-related sequence-A (MICA) and MICB in multiple myeloma cells, two ligands of the NK cell-activating receptor NK group 2 member D (NKG2D), by increasing MICA promoter activity and inhibiting MICB degradation in lysosomes, thereby improving NK cell-cytotoxicity [52].

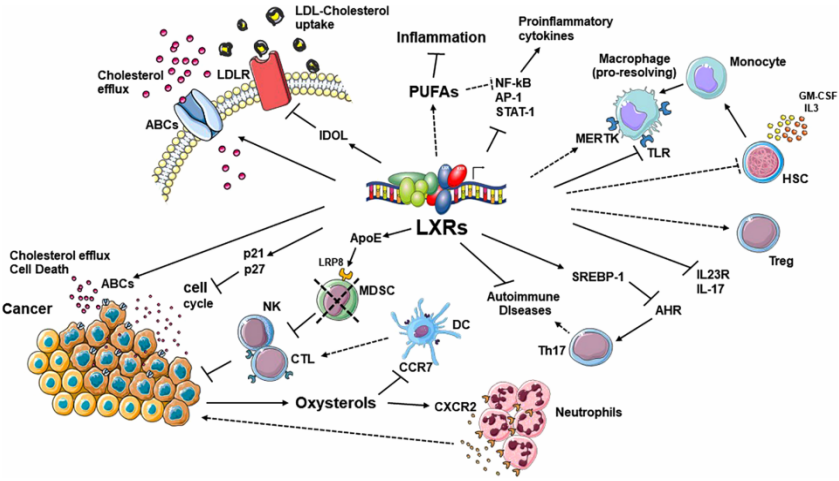


Figure 4. In the context of inflammation, autoimmunity, and tumour growth, a summary of the key pathways regulated by LXRs is provided. These NRs have cell-, tissue-, and context-specific actions. These regulatory mechanisms are further complicated by the numerous LXR-dependent and -independent pleiotropic effects mediated by oxysterols, which are produced in inflamed or tumoral microenvironments. AHR stands for aryl hydrocarbon receptor; APOE is for apolipoprotein E; CTL stands for cytotoxic T lymphocyte; DC, dendritic cell; IDOL, inducible degrader of the LDL-receptor; IRF3, interferon regulatory factor 3; LBD, ligand binding domain; PUFA, long-chain polyunsaturated fatty acid; LDLR, low-density lipoprotein receptor; LRP8, low-density lipoprotein receptor-related 8; MDSC, myeloid-derived suppressor cells; MERTK, MER proto-onc [53]

Inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase prevents mevalonate production from HMG-CoA and lowers mevalonate levels, and inhibits myocardial Rac1-GTPase activity to stifle NADPH oxidase activity and ROS generation are statins' most significant effects on lung cancer. Additionally, farnesyltransferase (FT) proteins, the RAF/ERK and AKT pathways, the EGFR, and the anti-apoptotic protein Mcl-1 are all inhibited by *statins* in both humans and animals. They also affect cellular energy homeostasis via activating LKB1 and AMPK, as well as the (p. 53) pathway and G1 cell cycle arrest, which prevent DNA damage [54].

The ROS (reactive oxygen species) inhibitor acetylcysteine (also known as N-acetyl-l-cysteine, NAC, or acetylcysteine) mitigates the effects of proteasome inhibitors. Additionally, it inhibits the synthesis of tumour necrosis factors. By inhibiting IB kinases, acetylcysteine (N-acetyl-l-cysteine) blocks TNF's ability to activate NF-B. Apoptosis is induced by acetylcysteine (N-acetyl-l-cysteine) through a mitochondria-dependent mechanism. Ferroptosis and viral replication are inhibited by acetylcysteine (N-acetyl-l-cysteine).

Milk thistle (Silybum marianum)

Different studies suggest that bile and liver conditions can be treated using a natural supplement called Milk Thistle (Silybum Marianum). Milk thistle is not subject to the same Food and Drug Administration (FDA) regulation and quality

control as standard pharmaceuticals because it is classed as a supplement rather than a drug.

The amount of active chemicals utilized can vary substantially depending on the production process and brand. There are currently many milk thistle tablets, capsules, and soft gels on the market, each with a different dosing recommendation.

While there is no defined correct dosage, the daily intake is a dosage that varies from 20 – 300 mg, which most people administer. If someone else is taking milk thistle for the liver, the suggested daily dose is 150 milligrams, taken one to three times each day.

This is a relatively high dose that can help with liver detoxification. It is recommended the assumption of 50 to 150 mg per day for continued use and liver support. It is best to take a high-quality product that contains between 50 and 150 mg of pure milk thistle extract in each capsule to modify the amount a patient takes based on her needs.

High cholesterol can cause cardiac problems and raise a person's risk of stroke. Milk thistle has been linked to lower blood cholesterol levels in certain studies, although the evidence is not conclusive.

According to a 2006 study [62], milk thistle may help lower cholesterol levels. It discovered that patients taking milk thistle to treat diabetes had lower cholesterol levels than those taking a placebo.

In a study made in 2016, the researchers fed rats a high-cholesterol diet [63], and they administered silymarin (the active ingredient in milk thistle) to rats at doses of 300 and 600 milligrams per kilogram (mg/kg) of body weight and discovered that the rats had considerably reduced total blood cholesterol and triglyceride levels. According to the results of this study, Silybin therapy significantly reduced serum total cholesterol (24%), triglycerides (21%), and LDL–C (24%). Silybin (300 and 600 mg/kg) significantly increased hepatic HDL–C while decreasing other lipid profiles in rats.

At both doses, Silybin dramatically reduced serum and hepatic total cholesterol, triglycerides, VLDL–C, and LDL–C while increasing HDL–C.

Several research on the use and good benefits of silybin marianium in mice have shown that this product, in addition to lowering cholesterol, has a positive influence on insulin secretion and anti-inflammatory properties.

The use of silybin marianium may be one of the mechanisms responsible for its effects against liver damage and insulin resistance [57].

Different studies have been performed to highlight the health benefits of milk thistle, some of them are even focused on the prevention or treatment of cancer, different types of cancers.

These researches confirm that milk thistle may be helpful in the prevention of the spread of some types of cancer.

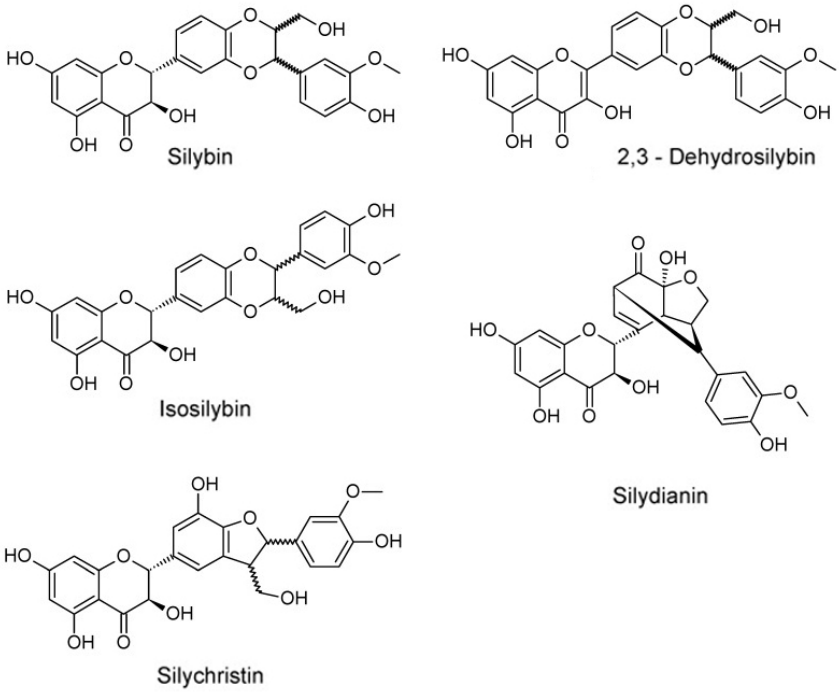


Figure 5. The structures of Silybin and its derivates[1]

According to the National Institutes of Health (NIH), small studies primarily conducted on cells in laboratories have discovered that milk thistle can reduce cancer cell and tumour growth and possibly boost the efficiency of chemotherapy. Researchers discovered advantages for a variety of cancers, including prostate cancer, breast cancer, cervix cancer, leukaemia, cancer of the skin, colon and rectal cancer [64].

[1] Molecules. 2019 Mar; 24 (6): 1022, Published online 2019 Mar 14. doi: 10.3390/molecules24061022

Several small studies have also discovered that milk thistle may reduce the negative effects of cancer treatments such as radiation, surgery, and chemotherapy, however, silymarin has not yet been approved by the Food and Drug Administration (FDA) for cancer treatment. More research is needed to establish how milk thistle can help fight cancer.

Silymarin, a combination of flavonoid complexes, is the main component that shields liver and kidney cells against the negative effects of medications, including chemotherapy [56]. It is important to emphasise that this component has also anti-inflammatory properties and it can lower the liver enzyme levels. Numerous research has validated the use of this natural product and its actual effectiveness in the suppression of liver cancer cells because of its anticancer and anti-inflammatory properties [58].

A new supercritical fluid extraction technique using carbon dioxide made it possible to conduct the first extraction and obtain the initial chemical profile of the Albanian Milk Thistle.

First-pass analyses revealed the presence of other substances with anti-inflammatory properties, as well as second metabolites that are thought that in combination with silymarin (which can be obtained in a second extraction), they may be a natural alternative for the suppression of liver tumour cells.

This is only a single perspective, but it suggests how silymarin characteristics and statin drugs combined can offer many people a good therapeutic alternative.

MATERIALS AND METHODS

With a new and innovative method such as SC–CO_2 extraction, a green technology, we were able to identify silybin derivatives, with anti-inflammatory and anti-tumoral properties

Materials

The plant, milk thistle,
n-hexane,
The SC CO2 extractor,
The GC -FID,
The

Method

The plant was harvested in Albania's Elbasan region, cleaned of any physical contaminants, and then treated to SC CO_2 extraction.

CO_2 has excellent solvent characteristics that can be easily adjusted by adjusting the pressure and temperature. Furthermore, at the supercritical state, carbon dioxide has an extremely low viscosity and no interfacial tension. As a result, it may easily penetrate complex structures and materials. Because of these characteristics, it is appropriate for a wide range of processes.

The procedure begins with the working tank, where the CO_2 for the operation is stored. It is then compressed and heated to the extraction temperature using a high-pressure pump. It is then guided into one of the extraction jars containing the carrier substance. The extractors have rapid opening closures for convenient and safe filling and discharging.

Solid raw materials are normally supplied to the extractor via a container. Several extraction containers can be coupled into an operational system to enable continuous extraction despite the extractors' batch operation. After exiting the extractors, the loaded carbon dioxide reaches the separators, where a change in pressure and/or temperature creates conditions in which the extract separates from the carbon dioxide. The extract can then be collected at the bottom of the separators. This is also where the solvent characteristics of CO_2 enable for fractionation of the extract into distinct separators. In the final separator, the CO_2 is normally expanded to a gas state to remove any residual chemicals.

This principle was performed even with the extraction of the milk thistle. This pressure level is critical because high-energy recompression should be avoided. Following separation, the gas is liquefied in a condenser and returned to the working tank. It can now be used once more. The container was tightly closed and the herb was kept in a well-ventilated area. The method utilized has some unique usage requirements, such as the requirement that the plant have a humidity of almost 6%.

After inserting the plant into the extractor, the plant's pure extract was extracted. Various herbs are extracted using the inert gas carbon dioxide. At 1071 PSI and 31.1 °C, the carbon dioxide molecule enters in a supercritical state; at this point, it possesses both liquid and gaseous properties. Such a situation is advantageous because CO_2 can diffuse and function as a liquid solvent in addition to reaching small places as a gas.

To achieve the necessary extract, inert gas molecules pass through the plant, the extract is then collected in a container, and the gas is recollected to be utilized once more. To determine the chemical profile of the extract, it was diluted with hexane in a ratio of 1:2. It is crucial to emphasize that this is only the first step in the extraction process; silybin (silymarin) must be extracted in two steps [59].

RESULTS AND DISCUSSIONS

The chemical profile of this essential oil was examined using gas chromatography and a flame detector. (The extract was previously diluted (1 to 2) using hexane. The table below shows the data that were obtained.

With the aid of HPLC, it was possible to determine the difference between volatile compounds and secondary metabolites.

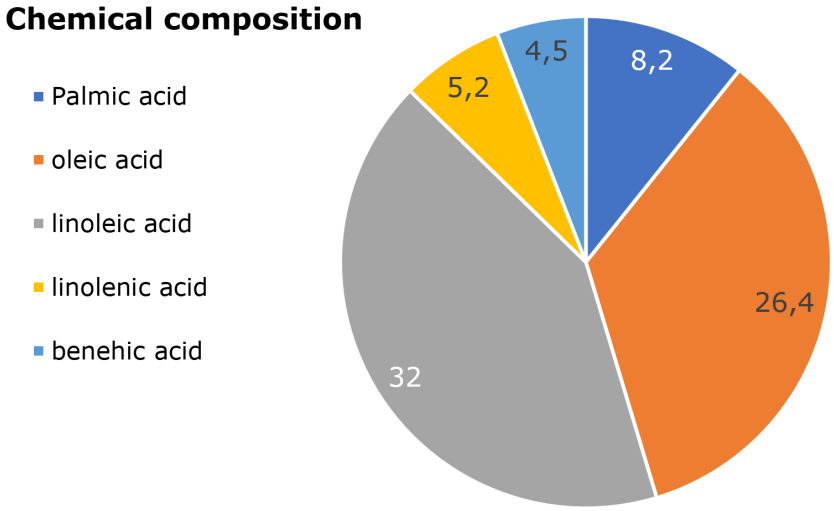
Table 1. The milk thistle essential oil components

	Compounds	Percentage
1.	Palmilic acid	8.2
2.	Oleic acid (omega 9)	26.4
3.	Linolic acid	32.0
4.	Linolenic acid (omega 6)	5.2
5.	Benehic acid	4.5

The results obtained, showed a high percentage of the oleic acid, at 26.4% and Linolic acid at 32%. Studies have revealed that the consumption of vegetable oils derives from both their greater availability and from the recommendations to consume these oils as a help to reduce blood cholesterol levels.

These studies have also shown that linoleic acid has also enabled the levels of cholesterol in the blood, but this does not provide for its effect on the development of atherosclerosis [60]. A high presence of oleic acid at 26.4%. in milk thistle

attributes health benefits to this natural product, which can protect against oxidative stress, endoplasmic reticulum (ER) stress, inflammation, and other health issues by activating several signaling pathways in hepatic parenchymal cells. Additionally, oleic acid lowers hepatocellular lipo-toxicity brought on by palmitic acid by preventing pyroptosis and ER stress [61].



Graphic 1. The graphical presentation of chemical composition of milk thistle essential oil (first extraction)

Alpha-tocopherol, which is detected at 25 /gr extract, and carotenoids, which are detected at 33 /gr extract, were the subjects of another HPLC investigation.

Table 2. The secondary metabolites

Secondary metabolites	Results	Method
a-tocopherol	25 μ/gr extract	HPLC
Carotenoids	33 μ/gr ectract	HPLC

The immune system is strengthened with alpha-tocopherol, which also helps to avoid blood clots. Additionally, it aids in safeguarding cells from free radicals, which are incredibly reactive chemicals. The use of alpha-tocopherol in the prevention and treatment of particular types of cancer is a topic of research. It provides an antioxidant effect. Also known as vitamin E.[1]

Human meals include carotenoid pigments, particularly beta-carotene and lycopene, which are crucial for maintaining good health. It has been demonstrated that carotene quenches singlet oxygen and has strong antioxidant action. Studies have suggested that carotene may reduce the risk of cancer as a result.

[1] D-α-tocopherol polyethylene glycol succinate and Poloxamer 188 modified liposomal chrysin hydrogel for enhanced topical treatment of ultraviolet-induced skin photoaging damage

Xiang Wang, Xuefeng Hou, Yihan Wu, Jingwen Guo, Haichuan Tai, Yongtai Zhang, Nianping Feng. Arabian Journal of Chemistry, Volume 16, Issue 7, July 2023, 104822

DISCUSSIONS AND CONCLUSIONS

The entire purpose of this study was to make a revision of the literature on the action that statins have in inhibitory cholesterol and consequently their anti-carcinogenic action on liver cells.

But an interesting combination was perhaps the combination of the Silybum marianum (milk thistle), which contains silybin, and consequently, the presence of this main compound and its derivatives can be combined and can fight (minimize the exposure of liver cancer cells). Numerous studies that refer to statins and their mechanism of action have been presented for a revision of their mechanism for the inhibition of cancerous cells, a laboratory study to extract Silybin derivatives, with a friendly environment method, such as SC CO_2 extraction method, allowed us to acquire a chemical profile of this compound and analyze secondary metabolites.

However, other investigations and studies are still needed, which can be a good opportunity so that these two compounds can also be combined to help prevent liver cancer cells in one.

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REFERENCES

1. Statin Toxicity-Mechanistic Insights and Clinical Implications-Natalie C. Ward, Gerald F. Watts and Robert H. Eckel. Originally published 17 Jan 2019. URL: https://doi.org/10.1161/CIRCRESAHA.118.312782. Circulation Research. 2019; 124: 328–350.
2. Lambert A. W., Pattabiraman D. R., Weinberg R. A. Emerging Biological Principles of Metastasis. Cell. 2017; 168: 670–91. URL: https://doi.org/10.1016/j.cell.2016.11.037
3. Nieto M. A. Epithelial plasticity: a common theme in embryonic and cancer cells. Science. 2013; 342:1234850. URL: https://doi.org/10.1126/science.1234850
4. Aceto N., Bardia A., Miyamoto D. T., Donaldson M. C., Wittner B. S., Spencer J. A., Yu M., Pely A., Engstrom A., Zhu H., Brannigan B. W., Kapur R., Stott S. L., et al. Circulating tumour cell clusters are oligoclonal precursors of breast cancer metastasis. Cell. 2014; 158: 1110–22. URL: https://doi. org/10.1016/j.cell.2014.07.013
5. Chao Y., Wu Q., Acquafondata M., Dhir R., Wells A. Partial mesenchymal to epithelial reverting transition in breast and prostate cancer metastases. Cancer Microenviron. 2012; 5:19–28. URL: https://doi.org/10.1007/s12307-011-0085-4

6. Taylor D. P., Wells J. Z., Savol A., Chennubhotla C., Wells A. Modeling boundary conditions for balanced proliferation in metastatic latency. Clin Cancer Res. 2013; 19: 1063–70. URL: https://doi.org/10.1158/1078-0432.CCR-12-3180
7. Wells A., Griffith L., Wells J. Z., Taylor D. P. The dormancy dilemma: quiescence versus balanced proliferation. Cancer Res. 2013; 73: 3811–16. URL: https://doi.org/10.1158/0008-5472. CAN-13–0356.
8. 'Statin-induced GGPP depletion blocks macropinocytosis and starves cells with oncogenic efects'. Zhihua Jiao, Huaqing Cai huaqingcai@ibp.ac.cn, Yu Long, and Peter N. Devreotes. – 117 (8). 41584168. URL: https://doi.org/10.1073/pnas.1917938117
9. Statins use and the prognosis of colorectal cancer: a meta-analysis, Liusheng Li, Ning Cui, Tengteng Hao, Jianhua Zou, Wu Jiao, Kangjun Yi, Wu Yu, PMID: 33662632. DOI: 10.1016/j.clinre.2020.101588.
10. 'Concomitant attenuation of HMG-CoA reductase expression potentiates the cancer cell growth-inhibitory effect of statins and expands their efficacy in tumor cells with epithelial characteristics'. Takuro Ishikawa, Yoshinao Z. Hosaka, Colin Beckwitt, Alan Wells, Zoltán N. Oltvai, and Katsuhiko Warita.
11. 'Mevalonate Pathway'. Heidrun Karlic, Franz Varga, in Encyclopedia of Cancer (Third Edition), 2019.

12. Ding X., Zhang W., Li S., Yang H. The role of cholesterol metabolism in cancer. Am J Cancer Res. 2019; 9: 219–227. [PMC free article] [PubMed] [Google Scholar] [Ref list].
13. Fatehi Hassanabad A. Current perspectives on statins as potential anti-cancer therapeutics: clinical outcomes and underlying molecular mechanisms. Transl Lung Cancer Res. 2019; 8: 692–699. [PMC free article] [PubMed] [Google Scholar] [Ref list].
14. Stryjkowska-Góra A., Karczmarek-Borowska B., Góra T., Krawczak K. Statins and cancers. Contemp Oncol (Pozn) 2015; 19: 167–175. [PMC free article] [PubMed] [Google Scholar] [Ref list].
15. Statins: a repurposed drug to fight cancer-Wen Jiang, Jin-Wei Hu, Xu-Ran He, Wei-Lin Jin, and Xin-Yang He. Journal List, J Exp Clin Cancer Res, – V. 40; 2021, PMC 8306262.
16. 'Statins Are Associated With a Decreased Risk of Decompensation and Death in Veterans With Hepatitis C-Related Compensated Cirrhosis'. Arpan Mohanty, Janet P. Tate, Guadalupe Garcia-Tsao, PMID: 26484707, PMCID: PMC4727998. DOI: 10.1053/j.gastro.2015.10.007.
17. Lipophilic Statins and Risk for Hepatocellular Carcinoma and Death in Patients With Chronic Viral Hepatitis: Results From a Nationwide Swedish Population. Tracey

G. Simon, Ann-Sofi Duberg, Soo Aleman, Hannes Hagstrom, Long H. Nguyen, Hamed Khalili, Raymond T. Chung, Jonas F. Ludvigsson. Affiliations expand, PMID: 31426090. PMCID: PMC8246628. DOI: 10.7326/M18-2753.

18. Statin Use and the Risk of Hepatocellular Carcinoma: A Meta-Analysis of Observational Studies, Md. Mohaimenul Islam, Tahmina Nasrin Poly, Bruno Andreas Walther, Hsuan-Chia Yang, and Yu-Chuan (Jack) Li.
19. Statins as Anticancer Agents in the Era of Precision MedicineJoseph Longo, Jenna E. van Leeuwen, Mohamad Elbaz; Emily Branchard; Linda Z. Penn- Clin Cancer Res. 2020. Nov. 15; 26(22): 5791-5800. Doi 10.1158/1078-0432.CCR-20-1967.
20. Even Cancer Cells Watch Their Cholesterol' Romain Riscal, Nicolas Skuli, M. Celeste Simon, PMID: 31586545. PMCID: PMC7225778. DOI: 10.1016/j.molcel.2019.09.008).
21. Ko, Y. J. & Balk, S. P. Targeting steroid hormone receptor pathways in the treatment of hormone-dependent cancers. *Curr Pharm Biotechnol* (2004). 5; 459–70.
22. Liver X. Receptors: Regulators of Cholesterol Metabolism, Inflammation, Autoimmunity, and Cancer- Maria Teresa Bilotta. Doi: 10.3389/fimmu.2020.584303. PMID: 33224146.

23. Lin, C. Y. & Gustafsson, J. A. Targeting liver X receptors in cancer therapeutics. *Nat Rev Cancer,* (2015). 15; 216–24.
24. Krycer, J. R. & Brown, A. J. Cholesterol accumulation in prostate cancer: a classic observation from a modern perspective. *Biochim Biophys Acta,* (2013). 1835; 219–29.
25. Murai T. Cholesterol lowering: role in cancer prevention and treatment. Biol Chem (2015). 396(1): 1–11. Doi: 10.1515/hsz-2014-0194.
26. Lo Sasso G., Bovenga F., Murzilli S., Salvatore L., Di Tullio G., Martelli N., et al.'Liver X receptors inhibit proliferation of human colorectal cancer cells and growth of intestinal tumours in mice. Gastroenterology (2013). 144(7): 1497–507, 1507 e1–13. Doi:10.1053/j.gastro.2013.02.005.
27. Bovenga F., Sabba C., Moschetta A. Uncoupling nuclear receptor LXR and cholesterol metabolism in cancer. Cell Metab (2015). 21(4): 517–26. Doi 10.1016/j.cmet.2015.03.002.
28. Vedin L. L., Gustafsson J. A., Steffensen K. R. The oxysterol receptors LXRalpha and LXRbeta suppress proliferation in the colon. Mol Carcinog, (2013). 52 (11): 835–44. Doi 10.1002/mc.21924.
29. Lin C. Y., Gustafsson J. A. Targeting liver X receptors in cancer therapeutics. Nat Rev Cancer, (2015). 15(4):216–24. Doi 10.1038/nrc3912.

30. Horton J. D., Goldstein J. L., Brown M. S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest, (2002). 109(9): 1125–31. Doi: 10.1172/JCI15593.
31. Yang J., Wang L., Jia R. Role of de novo cholesterol synthesis enzymes in cancer. J Cancer, (2020). 11(7): 1761–7. Doi 10.7150/jca.38598.
32. Iannelli F., Lombardi R., Milone M.R., Pucci B., De Rienzo S., Budillon A., et al. Targeting Mevalonate Pathway in Cancer Treatment: Repurposing of Statins. Recent Pat Anticancer Drug Discov (2018). 13(2):184–200. Doi 10.2174/1574892812666171129141211.
33. Guo D., Reinitz F., Youssef M., Hong C., Nathanson D., Akhavan D., et al. An LXR agonist promotes glioblastoma cell death through the inhibition of an EGFR/AKT/SREBP-1/LDLR-dependent pathway. Cancer Discov (2011). 1 (5):442–56. Doi 10.1158/2159-8290. CD-11–0102.
34. 'Targeting SREBP-1-Mediated Lipogenesis as Potential Strategies for Cancer', - Qiushi Zhao, Xingyu Lin, Guan Wang, REVIEW article Front. Oncol., 14 July, 2022. Sec. Molecular and Cellular Oncology, Volume 12–2022.
URL: https://doi.org/10.3389/fonc.2022.952371
35. Lewis C. A., Brault C., Peck B., Bensaad K., Griffiths B., Mitter R., et al. SREBP maintains lipid biosynthesis

and viability of cancer cells under lipid- and oxygen-deprived conditions and defines a gene signature associated with poor survival in glioblastoma multiforme. Oncogene, (2015). 34(40): 5128–40. Doi: 10.1038/onc.2014.439.

36. Villa G. R., Hulce J. J., Zanca C., Bi J., Ikegami S., Cahill G. L., et al. An LXR Cholesterol Axis Creates a Metabolic Co-Dependency for Brain Cancers. Cancer Cell, (2016). 30(5): 683–93. Doi: 10.1016/j.ccell.2016.09.008.
37. Chuu C. P., Lin H. P. Antiproliferative effect of LXR agonists T0901317 and 22 (R)-hydroxycholesterol on multiple human cancer cell lines. Anticancer Res, (2010). 30(9): 3643–8.
38. Chuu C. P., Hiipakka R. A., Kokontis J. M., Fukuchi J., Chen R. Y., Liao S. Inhibition of tumor growth and progression of LNCaP prostate cancer cells in athymic mice by androgen and liver X receptor agonist. Cancer Res. (2006). 66 (13): 6482–6. Doi 10.1158/0008-5472.CAN-06-0632.
39. Fukuchi J., Kokontis J. M., Hiipakka R. A., Chuu C. P., Liao S. Antiproliferative effect of liver X receptor agonists on LNCaP human prostate cancer cells. Cancer Res. (2004). 64(21): 7686–9. Doi 10.1158/0008-5472.CAN-04-2332.
40. Lee J. H., Gong H., Khadem S., Lu Y., Gao X., Li S., et al. Androgen deprivation by activating the liver X receptor.

Endocrinology. (2008). 149(8): 3778–88. Doi: 10.1210/en.2007-1605.

41. Horton J. D., Goldstein J. L., Brown M. S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* 2002. 109: 1125–1131 [PMC free article] [PubMed] [Google Scholar] [Ref list].
42. York A. G., Bensinger S. J. Subverting sterols: re-routing an oxysterol-signalling pathway to promote tumor growth. J Exp Med (2013). 210(9):1653–6. Doi:10.1084/jem.20131335.
43. Dufour J., Viennois E., De Boussac H., Baron S., Lobaccaro J. M. Oxysterol receptors, AKT and prostate cancer. Curr Opin Pharmacol (2012). 12(6):724–8. Doi: 10.1016/j.coph.2012.06.012
44. Lin C.Y., Huo C., Kuo L. K., Hiipakka R. A., Jones R. B., Lin H. P., et al. Cholestane 3 beta, 5 alpha, 6 beta-triol suppresses proliferation, migration, and invasion of human prostate cancer cells. PloS One. (2013). 8(6): e65734. Doi: 10.1371/ journal.pone.0065734.
45. Villablanca E. J., Raccosta L., Zhou D., Fontana R., Maggioni D., Negro A., et al. Tumor-mediated liver X receptor-alpha activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. Nat Med. (2010). 16(1):98–105. Doi: 10.1038/nm.2074.

46. Subverting sterols: rerouting an oxysterol-signaling pathway to promote tumor growth- Autumn G. York and Steven J. Bensinger. J Exp Med, Vol. 210 (9). PMC3754867.
47. Raccosta L., Fontana R., Maggioni D., Lanterna C., Villablanca E. J., Paniccia A., et al. The oxysterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils. J Exp Med. (2013). 210(9): 1711–28. Doi: 10.1084/jem.20130440.
48. Baek A.E., Yu Y. A., He S., Wardell S. E., Chang C. Y., Kwon S., et al. The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. Nat Commun. (2017). 8(1): 864. Doi: 10.1038/ s41467-017-00910-z
49. Mc Donnell D. P., Park S., Goulet M. T., Jasper J., Wardell S. E., Chang C. Y., et al. Obesity, cholesterol metabolism, and breast cancer pathogenesis. Cancer Res. (2014). 74(18): 4976–82. Doi: 10.1158/0008-5472. CAN-14-1756.
50. Derangere V., Chevriaux A., Courtaut F., Bruchard M., Berger H., Chalmin F., et al. Liver X receptor beta activation induces pyroptosis of human and murine colon cancer cells. Cell Death Differ. (2014). 21(12): 1914–24. Doi: 10.1038/cdd.2014.117.
51. Tavazoie M. F., Pollack I., Tanqueco R., Ostendorf B. N., Reis B. S., Gonsalves F. C., et al. LXR/ApoE Activation

Restricts Innate Immune Suppression in Cancer. Cell (2018). 172(4): 825–40 e18. Doi: 10.1016/j.cell.2017.12.026.

52. Bilotta M. T., Abruzzese M. P., Molfetta R., Scarno G., Fionda C., Zingoni A., et al. Activation of liver X receptor up-regulates the expression of the NKG2D ligands MICA and MICB in multiple myeloma through different molecular mechanisms. FASEB J (2019). 33(8): 9489–504. Doi: 10.1096/fj.201900319R
53. Liver X. Receptors: Regulators of Cholesterol Metabolism, Inflammation, Autoimmunity, and Cancer-Maria Teresa Bilotta, Sara Petillo, Angela Santoni, and Marco Cippitelli, Sec. Cytokines and Soluble Mediators in Immunity. Volume 11–2020. URL: https://doi.org/10.3389/fimmu.2020.584303.
54. The role of statins in lung cancer – Fatemeh Amin Farzaneh Fathi, Željko Reiner, Maciej Banach, Amirhossein Sahebkar, Arch Med Sci. 2022; 18(1): 141–152 DOI: https://doi.org/10.5114/aoms/123225.
55. An Adaptation To Life In Acid Through A Novel Mevalonate Pathway-Jeffrey M. Vinokur, Matthew C. Cummins, Tyler P. Korman &James U. Bowie. Article number: 39737 (2016).
56. Advances in the use of milk thistle (Silybum marianum), Janice Post-White, Elena J. Ladas, Kara M. Kelly. PMID: 17548789. DOI: 10.1177/1534735407301632.

57. 'Silymarin improved diet-induced liver damage and insulin resistance by decreasing inflammation in mice'. Yu Guo, Suli Wang, Ying Wang, Tiehong Zhu. PMID: 27387273.
DOI: 10.1080/13880209.2016.1199042
58. Clinical applications of Silybum marianum in oncology. Heather Greenlee, Kathy Abascal, Eric Yarnell, Elena Ladas, PMID: 17548794.
DOI: 10.1177/1534735407301727.
59. Silibinin, found in milk thistle, protects against UV-induced skin cancer, January 30, 2013. University of Colorado Denver.
60. Linoleic Acid: A Nutritional Quandary, Ronald J. Jandacek, Healthcare (Basel). 2017 Jun; 5(2): 25.
Doi: 10.3390/healthcare5020025.
61. Zeng X., Zhu M., Liu X., Chen X., Yuan Y., Li L. et al. Oleic acid ameliorates palmitic acid induced hepatocellular lipotoxicity by inhibition of ER stress and pyroptosis. Nutr Metab (Lond) (2020). 17: 11. [PMC free article] [PubMed] [Ref list].
62. 'The efficacy of Silybum marianum (L.) Gaertn. (silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial'H Fallah Huseini. Larijani B., Heshmat R., Fakhrzadeh H., Radjabipour B., Toliat T., Mohsin Raza. PMID: 17072885.
DOI: 10.1002/ptr.1988

63. Effect of Silybin on Lipid Profile in Hypercholesterolaemic Rats, Senthil Gobalakrishnan. Sylvia Santhakumari Asirvatham, and Venkatraman Janarthanam, J Clin Diagn Res. 2016. Apr; 10(4): FF01–FF05. Published online. 2016. Apr 1. Doi: 10.7860/JCDR/2016/16393.7566.
64. URL: https://www.cancer.gov/about-cancer/treatment/cam/patient/milk-thistle-pdq#link/_9

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