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REVIEW ARTICLE

Benefits of Silymarin as an Immune and Growth Enhancer in Farmed Fish

Abd El-Alim F. Abd El-Alim[1], Abdelhakeem El-Murr[2*] and Tahsein Hasan[1]

[1]Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt
[2]Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, PO Box 44511, Zagazig, Sharkia, Egypt

*Correspondence: Corresponding author: Abdelhakeem El-Murr: Email: somailmohsen@gmail.com

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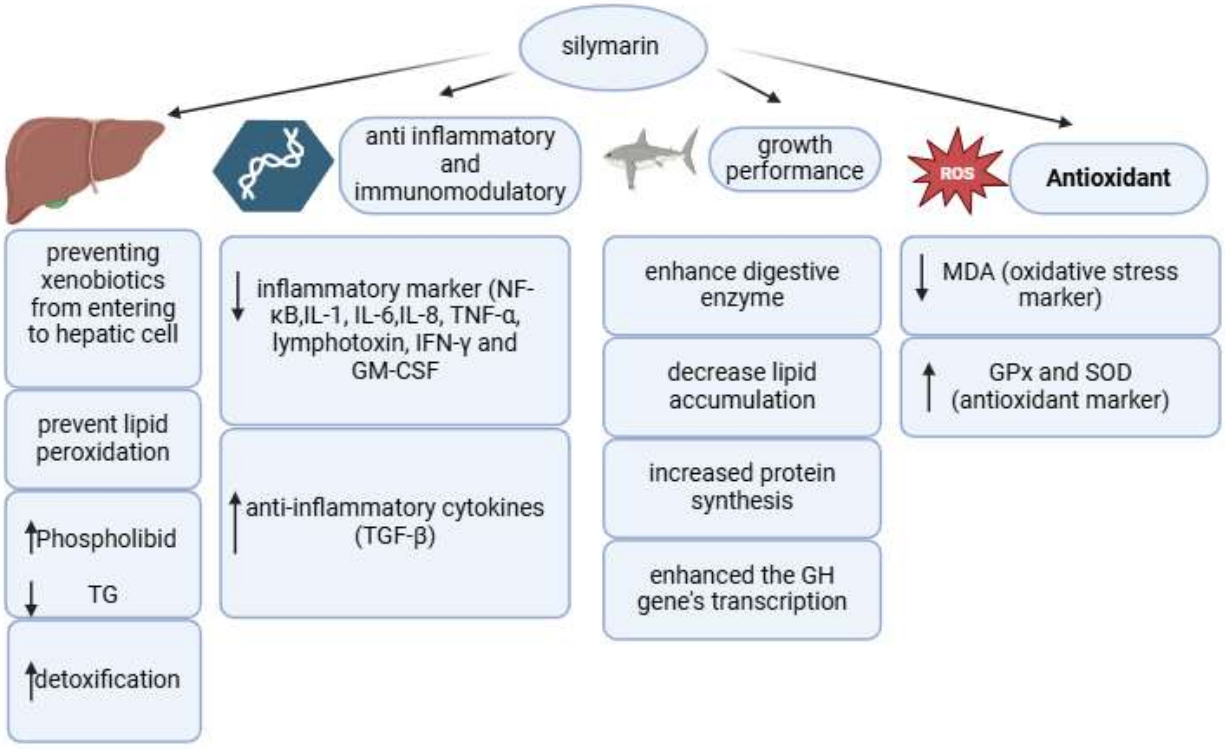
ABSTRACT

The potential advantages of silymarin, a flavonoid compound derived from milk thistle (Sillybum marianum) ((SM), for fish development and health have drawn more attention in aquaculture. This organic antioxidant is well-known for its immunomodulatory, hepatoprotective, and anti-inflammatory qualities. Supplementing fish with silymarin enhance liver function, detoxification, and defense against oxidative stress brought on by dietary toxins or environmental contaminants. Improved feed conversion ratios, quicker growth rates, and higher nutrient absorption all result from these impacts, which eventually raise production efficiency. There are various detrimental factors used in fish farming either used as feed additives or either introduced from polluted waters as drug residues, wastewater effluents and these molecules can lead to immune suppression and hepatotoxicity. Thus, the use of phytobiotics such as (SM), a source of silymarin, is receiving a lot of interest. The major flavonolignans of silymarin are in seeds with about 20%–30% of polymeric and polyphenolic compounds such as tannins. So, the various characteristics of silymarin such as its antioxidant, hepatoprotective, immunomodulatory, anti-inflammatory, and growth-promoting qualities are concerned in the current review.



Introduction

With increasing the idea that eating seafood is healthier than consuming other meats is a motive to increase the world's farmed fish production [1]. Fish is a fundamental and significant meal for human nutrition since it contains protein, good fats with low cholesterol that are safe to eat, and it can lower the risk of heart disease and stroke in addition to giving vital vitamins and minerals [2]. Due to the ever-growing human population, there is a constant need to increase aquaculture production in order to meet the need for fish protein. So, using intensive agricultural practices to boost production rates was the goal of so many farmers [3]. On the other hand, the intensity of production increase susceptibility to illness brought on by parasites, infections, and pests [4].

There are several serious disadvantages to using traditional drugs and vaccines for illness prevention and treatment [5]. Furthermore, using antibiotics to treat and prevent bacterial illnesses may cause microorganisms to become resistant to the drugs or the existence of leftover antibiotics in fish farmed for human need [6]. Therefore, we substituted synthetic feed additives and antibiotics with plant extracts rich in phytochemicals that have a high level of systemic bioactivity [7].

Since many medicinal plant extracts include antibacterial, antiviral, antiparasitic, antioxidant, anti-inflammatory, and immunostimulant qualities, they may be a potential way to increase farm animal productivity and to replace veterinary medications [8].

Phytobiotics are plants or plant extracts that can be added to aquafeed as

supplements to help fish grow more quickly, develop stronger immune systems, increase their level of antioxidants, and become more resistant to disease [9]. *Silybum marianum* (SM) is a type of phytobiotic feed supplement [9].

Silybum marianum (SM) and Silymarin

Silybum marianum (SM), sometimes known as milk thistle (MT), is a well-known herbal remedy plant from the *Asteraceae* family. is widely accessible, reasonably priced, and has no detrimental effects on fish or the surrounding environment [10]. For almost 2,000 years, MT seeds have been used medicinally, mostly to treat liver conditions [11]. MT is a tall, biennial herb that can grow up to 10 feet with big, thorny leaves, strong spiking stems and big purple blooming heads. The plant present in Kashmir, southern and Western of Europe and America [12]. The leaves typically measure between 50 and 60 cm in the length and 20 to 30 cm in the width [13]. Furthermore, a characteristic of the species is the white veins that run along the uppermost folio of the leaf [14].

Silymarin is a type of polyphenolic flavonoid that was isolated from milk thistle seeds using 95% ethanol. About 20–30% of the plant is made up of a chemically unknown fraction that is primarily composed of polymeric and oxidized polyphenolic chemicals, while the remaining 70–80% is made up of silymarin flavonolignans. The most active photochemical and the main contributor to the silymarin's putative benefits is silybin, which makes up 50–60% of the silymarin complex. In addition to silybin, which is a combination of two diastereomers (A and B) in a roughly 1:1 ratio, the silymarin complex contains significant levels of other flavonolignans, including silychristin (20%), silydianin (10%), isosilybin (5%), dehydrosilybin, and a few flavonoids, including taxifolin. Furthermore, the seeds include important fatty acids, trimethylglycine, and betaine, which may help justify silymarin's anti-inflammatory and hepatoprotective properties [15–17].silymarin flavonolignans, which have a variety of special biological properties including immunomodulatory, antioxidant, anti-inflammatory, and liver-regenerating properties [9,18-20]

Origin and Dispersal of MT

The milk thistle is indigenous to the Mediterranean basin, which includes a wide region that extends from southern Europe to Asia Minor and northern Africa, despite this, it has also become native in other parts of the world [21–24]. This species is distinctive of the Mediterranean-Turanic chorotype [22]. It is found throughout Italy, ranging from 0 to 1100 meters above sea level, with the notable exception of the Alps, Friuli, and the majority of the Po Valley [25]. Today, the plant is found all throughout the world [13], both as a crop [26] and in wild populations [27].

Pharmacodynamic of silymarin

The chemical structure of silybin is very hydrophobic and nonionizable, which makes it poorly soluble in water and has a limited bioavailability [28], because silybin's quick and extensive phase II metabolism (the main reason of this low bioavailability) [29]. Nevertheless, a number of variables, such as the presence of companion molecules like flavonoids, phenol derivatives, amino acids, and several other compounds, can affect silybin bioavailability [30]. There are several methods can be used to increase silybin's systemic bioavailability, such as adding solubilizing agents to MT extracts using phosphatidylcholine,

mixing it with vitamin E and phosphatidylcholine, forming micelles with bile salt, and most importantly using the self-microemulsifying drug delivery system, which delivers hydrophobic drugs using a microemulsion [28, 31]. As the carrier protein, silymarin was carried bound to serum albumin [32].

Silybin and its flavonolignans are extensively metabolized, primarily by phase II metabolic mechanisms [33]. Silybin monoglucuronide, silybin diglucuronide, silybin monosulfate, and silybin diglucuronide sulfate are the products of conjugation processes that occur during phase II [34]. Both conjugated and free silymarin were quickly removed in vivo. Silybin's renal excretion, however, is little and only makes up 1% to 2% of the initial oral dosage given over a 24-hour period [35, 36]. C-7 and C-20 are the two main sites for glucuronidation. Stereoselective glucuronidation of silybin occurs, with silybin B glucuronidating more effectively at the C-20 location and silybin A glucuronidating similarly on both sites [37]. The secondary peak in the plasma concentration curve indicates that the pharmacokinetic behavior of silybin in vivo, like that of most flavonoids, shows an enterohepatic circulation, where the expelled glucuronidated silybin is reabsorbed after bacterial enzymatic breaking of β-glucosidic linkages [38].

Mode of action of silymarin

The following are some of the various ways silymarin works: 1-boosting the formation of DNA and RNA to increase the liver cells' capacity for regeneration Since silymarin contains steroid-like properties, it can change the hepatocyte's outer membrane, preventing xenobiotics from entering the cell (a notable example of this method is poisoning with Amanita mushrooms), 2- scavenging free radicals and boosting glutathione levels within cells so lipid peroxidation is inhibited, 3-Silymarin is altering cell membrane transporters and receptors, including TNF-α-dependent transporters, bile salt export pumps, organic anion uptake transporter peptides (OATP), and ABC transporters (P-gp) [39, 40], 4-anti-inflammatory properties, including as blocking the production of prostaglandins and leukotrienes, inhibiting Kupffer cells, stabilizing mast cells, and preventing neutrophil migration [16, 41–45], and 5-Improved liver detoxification by phase I detoxification inhibition [46, 47].

Hepatoprotective effect of silymarin in fish species

Humans frequently use acetaminophen/ paracetamol to treat pain and fever, and it can reach aquatic habitats through inappropriate disposal practices and wastewater effluents [48]. Either acute or prolonged exposure to acetaminophen causes oxidative stress and hepatotoxicity [49]. When taken in very high doses, APAP (paracetamol) causes severe liver damage. APAP's hepatotoxicity has been linked to the production of the highly reactive and harmful metabolite N-acetyl-p-benzoquinone imine (NAPQI), which results in the depletion of glutathione and oxidative stress [50].

The liver is essential to the body's detoxification processes because it metabolizes and gets rid of foreign toxins [51]. Fish liver injury can be caused by exposure to hepatotoxic medication residues, which can interfere with liver function [52]. Among their many negative effects, pharmaceutical leftovers can destroy cells [53], inflammatory response [54], oxidative damage to several organs, including liver cells [55], modification of

the liver enzymes' activity that is related to metabolic and detoxifying activities [56], or disruption of the gut flora [57]. All of these side effects have the potential to impair vital liver activities, including lipid metabolism, protein synthesis, and detoxification [58]. Fish health and metabolism as a whole may be affected systemically by this disturbance [59].

Silymarin is a promising hepatoprotective agent as it contributing to hepatic lipid reorganization (encourages phospholipid biosynthesis varying degrees depending on the conditions by restricting the enzymes that break down phospholipids and reduces the production of triglycerides) [60]. A variety of hepatoprotective drugs have been studied against carbon tetrachloride, which is known to have hepatotoxic attributes. Silymarin has been demonstrated to stop hepatotoxicity and lipid peroxidation brought on by carbon tetrachloride [61,62]. Since silymarin has strong hepatoprotective and cardioprotective properties against oxidative stress brought on by paracetamol, it holds promise as a treatment for oxidatively damaged liver and heart conditions [63].

It has been demonstrated that silymarin, a hepatoprotective antioxidant with anti-lipid and anti-inflammatory qualities, has hepatoprotective benefits in common carp [64].The steroid structure of silymarin may change the hepatic cell membrane by preventing xenobiotics from entering and capturing free radicals. This would raise glutathione concentrations inside the cell and prevent lipid peroxidation [65]. Lower serum AST and ALT levels were seen in Nile tilapia fed a diet higher in S. marianum content, according to a prior study [10]. These reductions in serum AST and ALT levels could be explained by silymarin's potent antioxidant action, which raises glutathione levels intracellularly and improves the body's ability to eliminate free radicals and limit lipid peroxidation. As a result, the release of liver enzymes into the bloodstream may be delayed and the cell membranes may be shielded [65, 66].

Immunomodulatory effect of silymarin in fish species

Oxytetracycline has been licensed by numerous governments for use as feed additive. Nevertheless, it is well recognized that oxytetracycline might impair immunity [67]. An additional essential metric for evaluating the health and nutritional value of feed ingredients is the immune system's response. It has been extensively documented that fish fed on diets high in plant protein can develop intestinal enteritis [68]. Plant extracts' roles and possible use as immunopotentiators have been thoroughly studied. silymarin has been shown to have both immunostimulatory and immunosuppressive properties [69].

Silymarin may impede the initiation of gene transcription linked to the inflammatory response, as well as the degradation of inhibitory kappa B (I-κB) and the transcription of NFKB1 (encoding NF-κB) into the nucleus [70]. The preceding investigation revealed that fish fed diets containing silymarin had lower levels of pro-inflammatory cytokines (IL-8, TNF-α) and higher levels of anti-inflammatory cytokines (TGF-β) in the turbot intestines. And also, histological alteration may result from the raised immune response of the intestine [71].

In the hepatic tissues of Nile tilapia fed dietary silymarin, there was an increase in IL-1β, TNF-α, and IL-10 levels; however, after eight weeks, the levels had returned to baseline [67]. Up to

10 mg/kg of silymarin may inhibit WBC function; greater doses (50–250 mg/kg) have been shown to incite inflammatory processes [40].

Micelle silymarin markedly enhanced innate immune responses, including lysozyme, anti-protease, myeloperoxidase, and total immunoglobulin than silymarin [72]. A recent study showed that giving SM 1 g/kg feed along with Berberine (BBR)100 mg/kg feed reduced the oxidative stress and altered the nonspecific immune system, indicating the nutraceutical combination's strengthening effect [73].

Silymarin and growth performance in fish species

To improve the diet's palatability and consequently the development and feed efficiency of Nile tilapia, supplements containing *S. marianum* extract could be added [74]. supplements containing silymarin improved growth performance and improved intestinal physical barrier function, as evidenced by the juvenile grass carp's improved intestine apparent shape and decreased intestinal mucosa permeability and also raised growth factors such feed intake (FI), feed efficiency (FE), percent weight gain (PWG), specific growth rate (SGR), and final body weight (FBW) [75]. Higher villi and enterocyte heights were seen when 100 or 200 mg/kg of silymarin was administered [71]. Silymarin enhance digestive enzyme activities may due to its beneficial role on fish intestinal morphology [71]. And also, decrease lipid accumulation through reduction of lipogenesis and promote lipolysis and this reflected by decrease expression of Sterol regulatory element-binding transcription factor 1 (srebp-1) and increase expression of pparα [76].

In common carp, *Silybum marianum* enhanced both growth performance and the function of the liver enzymes [77]. Fish fed diets supplemented with varying concentrations of silymarin had a substantially higher survival rate than fish fed a control diet [10]. Silymarin increased protein synthesis and retention while also enhancing fish growth and feed efficiency [78]. Furthermore, SM enhanced the GH gene's transcription, which might have helped fish muscle expand [10]. It is anticipated that dietary micelle silymarin will be a more cost-effective and efficient supplement for olive flounder than ordinary silymarin [72].

In large yellow croaker larvae, silymarin supplementation at a dose of 50 mg/kg SM may enhance growth performance, antioxidant capacity, and digestive enzyme activities while lowering visceral mass lipid accumulation [76]. For Nile tilapia, *O. niloticus* fingerlings, the optimal dietary *S. marianum* level was 7.5 g or 10 g kg−1 diet (92.25 and 123 mg kg−1 silymarin) as a feed additive to stimulate growth, improve immunological responses, boost antioxidant activity, and raise gene expression [10].

Antioxidant properties of silymarin in fish species

Similar to other organisms, fish are susceptible to DNA hydroxylation, protein denaturation, lipid peroxidation, apoptosis, and eventual cell death due to an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense system, a condition known as oxidative stress [79]. The primary biological acceptor of electrons, oxygen is essential to cellular processes. Nevertheless, despite its advantageous qualities, it promotes the unfavorable

development of ROS such superoxide, hydrogen peroxide, and radical hydroxyl [80]. The ways in which silymarin's antioxidant qualities work can vary. These include inhibiting the enzyme activity that generates reactive oxygen species, preventing the generation of free radicals, intestinal ion chelation, promoting the creation of chemicals that provide protection, and triggering antioxidant enzymes [81].

Silymarin is well-known for having antioxidant qualities and has been researched for possible defense against a range of pollutants and illnesses linked to oxidative stress [82]. It is believed that the antioxidant and radical scavenging properties of silymarin components are mediated by the presence of hydroxyl groups in their molecular structure. Consequently, by scavenging free radicals and regulating inflammatory cytokines, silymarin can mitigate the adverse effects of oxidative stress and the inflammatory process. Silymarin's in vitro antioxidant activity was achieved by combating the free radicals 2,2'-azino-bis (3-ethylbenzene-thiazoline-6- sulfonic acid diammonium salt) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) [83].

Through served as an antioxidant itself or may have strengthened the fish's natural antioxidant defense systems, such as Superoxide dismutase (SOD) activity [84]. Research findings indicate that silymarin has the ability to directly engage with ROS molecules and providing an electron to stabilize and stop them from doing harm [85]. Moreover, silymarin can promote the synthesis of endogenous antioxidants like glutathione, which aid in mitigating the negative effects of ROS [86]. In addition, it can attach metal ions like copper and iron, which can catalyze the production of reactive oxygen species (ROS) [87, 88]. It was also discovered that giving fish exposed to diazinon silymarin brought their Malondialdehyde (MDA) levels back to normal [89]. These MDA is a lipid peroxidation and oxidative stress marker that may indicate cell damage [90]. Silymarin's ability to scavenge radicals improves hepatic lipid homeostasis by inhibiting denovo lipogenesis by downregulating FAS (fatty acid synthase), ACC (acetyl-CoA carboxylase), and PPARs (peroxisome proliferator-activated receptor) [91].

Catalase (CAT) is an enzyme found in cells that helps break down hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage [92]. Moreover, silymarin may have balanced the equilibrium of antioxidant enzymes and decrease CAT activity in order to control the oxidative stress response [89]. It has been demonstrated that silymarin's antioxidant properties improve poly-(ADP-ribose)-polymerase function by preserving sirtuin 1 (SIRT1) activity, Nicotinamide Adenine Dinucleotide (NAD+) homeostasis, and the AMP-activated protein kinase pathway—all important regulatory mechanisms linked to oxidative stress [93].

It was reported that silymarin had hepatoprotective effects on Glutathione peroxidase (GPx) activity. it is a crucial antioxidant enzyme that lowers lipid hydroperoxides and hydrogen peroxide, thereby shielding cells from oxidative damage [94]. According to a recent study, silymarin extract at a dose of 1400–2400 mg/kg diet may improve antioxidant defense and shield hepatocytes from cadmium's harmful effects [95].

The toxicity of silver nanoparticles (AgNPs) was shown to be reduced by free silymarin (FS) or nanoencapsulated

silymarin (NS), however NS supplementation proved to be the most successful [96]. Recent study demonstrated that micelle silymarin has significantly higher antioxidant capacities as superoxide dismutase and glutathione peroxidase, and lower lipid peroxidation as malondialdehyde than silymarin [72].

The liver's antioxidant capability increases by Adding 100 or 200 mg/kg of silymarin to the diet as it not only inducing the activities of superoxide dismutase (*SOD*) and catalase but additionally raising the levels of SOD, peroxiredoxin 6, and glutathione peroxidase messenger RNA (mRNA) expression [71].

In fish, silymarin demonstrated promise as a medicinal remedy to reduce oxidative damage brought on by diazinon [89]. It is a common insecticide used in residential areas, livestock farmlands, and agricultural to control pests. However, its use has contaminated surface waters in the US and many other countries [97–99]. Researchers have discovered diazinon traces in lakes, rivers, and streams as a result of drainage from homes and farms [100, 101]. Diazinon exposure can upset the equilibrium between antioxidants and ROS in cells. Inhibition of the mitochondrial respiratory chain, activation of NADPH oxidase enzymes, and impairment of cellular antioxidant defenses are some of the ways that diazinon can produce ROS. This oxidative stress can harm lipids, proteins, and DNA, among other biological constituents [90,102,103].

Anti-inflammatory properties of silymarin in fish species

Silymarin is an immunomodulator that, at low concentrations, inhibits T-lymphocyte function and, at high concentrations, causes inflammation [69]. By suppressing the transcription factor (NF-κB), which is required for the synthesis of interleukins (IL-1, IL-6), tumor necrosis factor (TNF-α), lymphotoxin, interferon (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF), silymarin reduces inflammation [104]. Furthermore, it prevents TNF-α-induced NF-κB activation through preventing the phosphorylation and degradation of inhibitory protein IκBa. [105]. Moreover, it prevents c-Jun N-terminal kinase and mitogen-activated protein kinase from being activated by TNF-α and causes the activation of caspase-3 and caspase-9, the release of cytochrome c, the cleavage of poly (ADP-ribose) polymerase (PARP), and the suppression of cell development [106].

Conclusion

Recently silymarin used as feed additives in fish diet instead of various antibiotic, probiotic and vaccination due to its antioxidant, hepatoprotective, immunomodulatory, anti-inflammatory, and growth-promoting qualities, but further investigation and research required to validate these properties in various species of fish exposed to various detrimental factors and how silymarin intake induce these properties.

Conflict of Interest

The authors declare no conflict of interest.

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الملخص العربي

فوائد السيليمارين كمحسن للمناعة والنمو في الأسماك المستزرعة

عبدالعليم فؤاد عبدالعليم1، عبدالحكيم المر2* و تحسين حسن2
1 قسم الفارماكولوجيا، كلية الطب البيطرى، جامعة الزقازيق، مصر 44511
2قسم طب الأحياء المائية، كلية الطب البيطرى، جامعة الزقازيق، مصر 44511

اجتذبت المزايا المحتملة للسيليمارين، وهو مركب فلافونويد مشتق من شوك الحليب (Sillybum marianum)، لنمو الأسماك وصحتها المزيد من الاهتمام في تربية الأحياء المائية. يُعرف هذا المركب بخصائصه المضادة للأكسدة وتعزيزه للمناعة وحماية الكبد وأثره المضاد للالتهابات. تعمل المكملات الغذائية التي تحتوي على السيليمارين على تحسين وظائف الكبد وإزالة السموم والدفاع ضد الإجهاد التأكسدي الناتج عن السموم الموجودة بالغذاء أو الملوثات البيئية بالمياه. وتنجم عن استخدام المكملات الغذائية التي تحتوى على السيليمارين زيادة نسب تحويل الأعلاف ومعدلات النمو الأسرع وامتصاص العناصر الغذائية الأعلى، مما يؤدي في النهاية إلى زيادة كفاءة الإنتاج. هناك العديد من العوامل الضارة أثناء تربية الأسماك إما المستخدمة كإضافات للأعلاف أو إما يتم إدخالها من المياه الملوثة كبقايا الأدوية ومياه الصرف الصحي ويمكن أن تؤدي هذه الجزيئات إلى تثبيط المناعة وتسمم الكبد وبالتالي، فإن استخدام المواد الحيوية النباتية مثل (Sillybum marianum)، وهو مصدر للسيليمارين، يحظى باهتمام كبير. الفلافونوليجنينات الرئيسية في يحتوي السليمارين في البذور على حوالي 20% - 30% من المركبات البوليميرية والبوليفينولية مثل العفص. لذا، سيتم توضيح الخصائص المختلفة للسيليمارين مثل خصائصه المضادة للأكسدة، والحماية للكبد، وتعديل المناعة، والمضادة للالتهابات، وتعزيز النمو.