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MILK THISTLE - MORPHOLOGY, CHEMISTRY AND PHARMACOLOGICAL ACTION

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Abstract
Silymarin reveals extensive range of biological accomplishments, but none of them were overlooked. In this review the biological activity like anti-oxidant, anti-inflammatory, hepatoprotective, antidiabetic activity was explored. The aim of this article is to make biological activities more selective and projecting. About 200 derivatives of silymarin have been prepared in more than 50 years since the discovery of silymarin, and some derivative of silymarin is now clinically used. Silymarin conversely, has seen its accomplishment as extensively used nutraceutic and the production of silymarin is progressively intensifying. Synthetically and semisynthetically, structural and peripheral modifications, either in the form of skeletal rearrangements or partial degradations as well as functional group addition and replacement giving access to new structural motifs. An additional favourable prospect for the imminent lies in further refinement and scale up of the separation processes for silymarin diastereomers and other pure silymarin components.

Keywords: Milk thistle, Silybum marianum, Silymarin, Hepatoprotective, Anti-inflammatory, Antidiabetic.

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INTRODUCTION

Silybum marianum (Compositae/Asteraceae) is an annual or biennial thistle-like plant (Milk Thistle) (Figure 1) as a relatable point in the Mediterranean territory of Europe. The seeds yield 1.5–3% of flavonolignans, considerably termed as silymarin. This mixture holds primarily silybin, together with silychristin, silydianin, and little sum from claiming isosilybin. Both, silybin as well as isosilybin are equimolar mixtures which are of two trans diastereoisomers. *Silybum marianum* may be broadly utilized within accepted European medicine. The seeds constantly are utilized to treat an assortment of hepatic as well as other disorders. Silymarin has been indicated to conserve animal livers against the harming impacts of carbon tetrachloride, thioacetamide, medications for example paracetamol, and the toxins α-amanitin and phalloin discovered in the death cap fungus (*Amanita phalloides*). Silymarin might be utilized within huge numbers, for instance, in liver infection and injury. However, even now it stays subsist to standard medication. It could offer specific profit in the medication of poisoning, towards the death cap fungus. These agents show up on two fundamental modes of action. They follow up on that cell membrane of hepatocytes hindering absorption from claiming toxins. What's more secondly, due to their phenolic nature; they act as antioxidants as well as scavenger's free radicals which are harmful to liver. The silymarin start with liver detoxification to remove exotic chemicals. Subsidiaries about silybin with enhanced water-solubility or bioavailability have also been developed, e.g. The bis-hemisuccinate and phosphatidylcholine intricate [1].

Fig. 1: Milk Thistle Plant

VERNACULAR NAMES

Akùb, Artichnuat sauvage, blessed thistle, bull thistle, cardo blanco, cardo de burro, cardo mariano, carduo mariano, chardon argente, chardon-marie, épine blanche, Frauendistelfrüchte, fructus cardui mariae, fruit de chardon marie, holy thistle, kharshat barri, khorfeish,

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kocakavkas, kuub, Lady's milk, Lady's thistle, lait de Notre Dame, marian thistle, máriatövis-termés, mariazami, Mariendistel, Mariendistelfrüchte, Marienkörner, maritighal, mild marian thistle, milk thistle, pternix, shawkeddiman, Silberdistil, silybe, silybon, silybum, St Mary's thistle, thistle, thistle of the Blessed Virgin, true thistle, variegated marian thistle [2,3].

HISTORY

Milk thistle has been utilized since the time of antiquated doctors and botanists to treat an extend of liver and gallbladder clutters, counting hepatitis, cirrhosis, and jaundice, and to protect the liver against harming from chemical and natural poisons, counting wind nibbles, insect stings, mushroom harming, and alcohol. Milk thistle's history starts with its title. The scientific title for milk thistle is *Silybum marianum*: "Silybum" is the title that Dioscorides gave to consumable thistles [4] and "marianum" comes from the legend that the white veins running through the plant's leaves were caused by a drop of the virgin mary's milk [4-7]. As trying to find a place to nurture the newborn baby, Jesus when intending Egypt, Mary could only find shield in a bower shaped by the prickly leaves of the milk thistle [3]. From this story was born the folk belief that the plant was great for nursing mother [8]. Milk thistle may be a part of the aster or daisy family(Asteracea), which incorporates, in expansion to asters and daisies, a host of other thistles and the artichoke [9]. Milk thistle is one of the foremost imperative therapeutic individuals of this genus [10]. For centuries, about each portion of the plant, from root to hull has been utilized in some ways [7,8]. In her popular book, Maud Grieve clarified a few ways that the milk thistle can be eaten, including the heads like an artichoke, crude stalks (which are considered agreeable and nutritious), and the leaves as a salad [10]. She also cited Bryant, who wrote in his Vegetation Dietetica, "The young shoots in the spring, cut near to the root with portion of the stalk on, is one of the best boiling servings of mixed greens that's eaten, and outperforms the finest cabbage. The roots can be eaten like those of salsify [10]. Milk thistle has moreover verifiably been utilized for creature nourish. Grieve composed that in parts of England, the leaves are called "pig leaves" since pigs preferred them; moreover, the seeds are a favorite food of goldfinches [10]. In Scotland, the leaves were utilized broadly as nourishment for cattle and horses (the leaves were beaten and smashed to freed them of prickles) before the preface of spatial green crops [10]. The milk thistle prickles have also been utilized truly as a substitute for barbed wire [11]. In spite of this wide range of realize value, agriculturists considered it and other thistles a sign of untidiness and neglect [11]. Most sources on Milk thistle, which is local to southern Europe (particularly Mediterranean areas) and Asia [3], put its starting at 2000 a long time ago [4][5][8][11]. One of the most punctual notices of thistles in common is within the bible (Genesis 3:18). In this

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verse, God told to Adam and Eve when they were expelledfrom the Garden of Eden that "thorns also andthistles should it bring forward to there. A few of the most punctual individuals to utilize and inscribe about milk thistle were old Greek and Roman physicians and herbalists, each of whom appeared to have their possess title for the herb. Dioscorides called it "silybon", Pliny the senior called it "sillybum," and Theophrastus called it "pternix" [3]. Dioscorides' recommended planning it in a tea "for those that be chomped of serpents" [3]. Another famous antiquted botanist, Pliny the senior, inscribed that blending the juice of the plant with nectar was great for "carrying off bile" [4, 11].Milk thistle is said in a few works from the middle ages, one of primary being in a record of old Saxon remedies, which climed that "this wort if hung upon aman's neck it setteth snakes to flight" [10]. One of the finest known botanists from this time, John Gerard (1545-1612) recommended Milk thistle for expelling melancholy and its related infections [10,11,3]. Another well-known English botanist, Nicholas Culpeper (1616-54), recommended milk thistle for few illnesses: breaking and removing stones, expelling obstacles of the liver and spleen, treating jaundice and infections of the plague, and cleansing the blood [10,11].Maud Grieve, who compiled her book of herbal data in 1931, cited a few early English botanists on their love for and utilize of milk thistle. She reports that in 1694, Westmacott wrote of milk thistle, "It is a companion to the liver and blood: the prickles cut off, they were formerly utilized to be boiled in the spring and eaten with other herbs; but as the world decays, so does the utilize of grate ancient things and other more sensitive and less virtuous brought in" [10]. She also reports that John Evelyn inscribed, "Disarmed of its prickles and boiled it is worthy of esteem, and thought to be an extraordinary breeder of milk and appropriate diet for women who are nursing" [10]. In spite of the fact that milk thistle was utilized during the 17th century most frequently related with English herbalists, numerous religious communities developed and utilized the plant for therapeutic purposes as well. St.Hildegard Von Bingen (1098-1179) suggested that roots, herbs, and leaves for swelling and erysipelas [3]. Milk thistle was well known with German botanists and researchers, moreover. Otto Brunfels (1488-1534), Hieronimous Bock (1498-1554), Jacob Theodorus (1520-90), Adam Lonicerus (1528-86), and Pietro Andrea Mattioli (1501-77) have all suggested milk thistle for treating liver diseases [3,11]. Another German physician from the 19th century, Johannes Gottfried Rademacher, created a tincture made from milk thistle seeds for his liver patients [4,7]. Rademachers tincture is an ethanol extract from the seeds utilized for hepatosplenic disorders [11]. In the United States, milk thistle delighted in ubiquity in the 19th century with the Eclectics movement, a formally recognized department of North American medicine that transcendently used native American herbs. In the Eclectics

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utilized milk thistle for varicose veins, menstrual difficulty, and clog of the liver, spleen, and kidneys [4,9]. Milk thistle was moreover utilized as portion of the naturopathic therapeutic convention and local American medical practices [3]. Milk thistle was so popular that a tincture of the entire plant was recorded in the first United States Homeopathic Pharmacopoeia [4,5].Although milk thistle is most often related with treating liver disorders, physicians have attempted to apply its curative properties to other sicknesses, counting invigorating breast-milk generation and bile secretion, treating depression, and ensuring against the noxious mushroom (*Amanita Phalliodes*) and other natural toxins [7].

IDENTIFICATION AND MORPHOLOGICAL CHARACTERS OF THE PLANT

Milk thistle is a tall, biennial herb with a height of five to ten feet, hard, green, shiny leaves with spiny edges and white streaks along the veins. The solitary floral heads are reddish-purple and the bracts end in sharp spines (Figure 2). The small hard fruit in the flowers, technically known as achenes, looks like seeds and is part of a medicinally used plant (Figure 3). Milk Thistle is grown in South and Western Europe, South America and North America in eastern USA and California.Dried seeds contain 1-4% flavonoids of Silymarin [3]. Silymarin is a mixture of three or more flavonolignans, including silybin (silibinin), silidianin and silychristine. These are the main active ingredient in milk thistle and can also be found in related species like artichokes. dehydrosilybin, deoxysilycistin, deoxysilydianin, silandrin, silybinome, silyhermin and neosilyhermin are other flavonolignans identified in *S. marianum*. Moreover, milk thistlecontains apigenin; silybonol; myristic, oliec, palmitic and stearic acids; and betain hydrochloride, which can have a hepatoprotective effect [12].

Fig.2: Milk Thistle Seeds (Fruits)

Fig.3: Milk Thistle Leaves.

CHEMICAL CONSTITUENTS

Silymarin accounts for 1.5–3 percent of the dry weight of the fruit and is an isomeric mixture of unique flavonoid-flavonolignan complexes. Silymarin, isosilybin, slychristin,

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isosilychristin, silydianin (Figure 4) and silimonin are the main representatives of this group [13-18]. The chemical composition of milk thistle fruit in addition to flavonolignans also include other flavonoids (such astaxifolin, quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodyctiol, and chrysoeriol), 5,7-dihydroxy chromone, dehydroconiferyl alcohol, fixed oil (60% linoleic acid; 30%, oleic acid;9% palmitic acid), tocopherol, sterols (cholesterol, campesterol, stigmasterol, and sitosterol),sugars (arabinose, rhamnose, xylose, and glucose), and proteins [13]. The highest concentration of silybin, which comprises approximately 50-70 percent of the extract, is the main bioactive component of the extract, which has been confirmed in several studies. The concentrations of silybin typically found in common pharmaceuticals with a silymarin range of 20-40 percent[18].In addition to the hepatoprotective action, silybin has strong antioxidant properties and modulates a variety of cell signaling pathways, reducing pro-inflammatory mediation[19]. Silybin is also studied as an anti-cancer and chemotherapy agent [18]. Research has shown that silybine can inhibitserine proteases associated with the blood coagulation process [20,21], and also reduce the response of blood platelets to physiological agonists[22-25].

SILYBIN STRUCTURE AND CHEMISTRY

In 1968, Pelter and Hansel first established the chemical structure of silybin by careful examination of ^{1}H-NMR (100 MHz, DMSO-d6) and MS spectra [26]; However, the absolute configuration of silybin in positions C-2 and C-3 was found by the same researchers using a degrading method in 1975 [27]. Silybin, also referred to as flavobin, silliver, silybin, silymarin I, silybin and silybin, has a $C_{25}H_{22}O_{10}$ molecular formula and a molecular weight of 482.441, CAS No. 22888-70-6 (data obtained from the pubchem website).

The structure of silybins consists of two main units. The first is based on a taxifolin, which is a flavononol group in flavonoids. The second is a phenyllpropanoid unit, which is conyferil alcohol in this case. These two units are connected to a single structure by an oxeran ring (Figure 5) [28-30]. Silybin has weak acidic properties in neutral aqueous solutions, with pKa of 6.63 for 5-OH group, 7.7-7.95 for 7-OH group and 11.0 for 20-OH group [31,32]. In the structure of silybin, we can identify five hydroxyl groups, which are the main goals of the derivation process. Three hydroxyl groups (5-OH, 7-OH and 20-OH) have a phenolic character. The 5-OH group has a very strong hydrogen bonding to the adjacent oxo group in conjunction with the aromatic ring and acts as a free electron pair donor to the 5-OH group. The 7-OH and 20-OH have similar properties, although the C-7 OH group is more reactive than the 20-OH group because of its lower steric barrier and the presence of a hydrogen bond. The C-23 OH group has properties that lead to carboxylic esterisation or oxidation. The C-3

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OH group can easily be oxidized to a ketone (even with atmospheric oxygen) that produces 2,3-dehydrosilybin. Silybin is poorly soluble in polar protic solvents (EtOH and MeOH) and insoluble in non- polar solvents (chloroform and petroleum ether), but highly soluble in polar aprotic solvents like DMSO, acetone, DMF and THF [30].

Silybin is found in nature as two trans diastereoisomers: A and B (Figure 6). These two diastereoisomers are differentiated with respect to reference positions C-10 and C-11 in the 1,4-benzodioxane ring [28,33]. Silybin A and silybin B both have ^{1}H and ^{13}C NMR spectra, which are very similar (without any characteristic signals), and impede the detailed identification of individual isomers [34]. The most popular method for separating these two diastereoisomers is high-performance liquid chromatography (HPLC), which can distinguish the molecules by analyzing the retention time [35]. Despite problems with NMR spectra, these diastereoisomers absolute configurations have been established as follows: Silybin A is 2R, 3R, 10R, 11R isomer with a proper IUPAC name of (2R,3R)-2-[(2R,3R)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benzodioxin-6-yl]-2,3 dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one. Silybin B has a configuration of 2R, 3R, 10S, 11S and IUPAC name (2R,3R)-2-[(2S,3S)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benzodioxin-6-yl]-2,3-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one [34,36,37]. Silybin diastereoisomers also have different parameters of optical rotation:silybin A has an $[\alpha]_D^{23}$ +20.0° (c 0.21, acetone), whereas silybin B has an$[\alpha]_D^{23}$ -1.07° (c 0.28, acetone) [37].

After compound crystallisation, the differences between silybin A and silybin B can also be observed. Silybin A (MeOH–H2O) forms yellowish flat crystals with a melting point of 162–163°C, while silybin B generates yellow grain crystals (MeOH–H2O) with a melting point of 158–160° C [34].

Flavonolignans form in *Silybum marianum* by combination of flavonoid and lignan structures.This occurs through the oxidative coupling of flavonoids and a phenylpropanoid. Freudenberg described the first hypothesis of this process [38] and Hansel confirmed [39]. Biosynthesis of Silybin from (+)- taxifolin and coniferyl alcohol is an oxidative process, catalyzed by peroxidase enzyme [40]. The first step in this process involves the single electron oxidation of substrates, which leads to the generation of free radicals derived from taxifoline and coniferyl alcohol.These individuals react in the O-β coupling stage, which leads to the formation of an addict. The adduct subsequently undergoes cyclization by adding the phenol nucleophile to the coniferyl alcohol-generated quinine methide.The formation of two diastereoisomers shows that the radical coupling reaction is not stereospecific [40-42].

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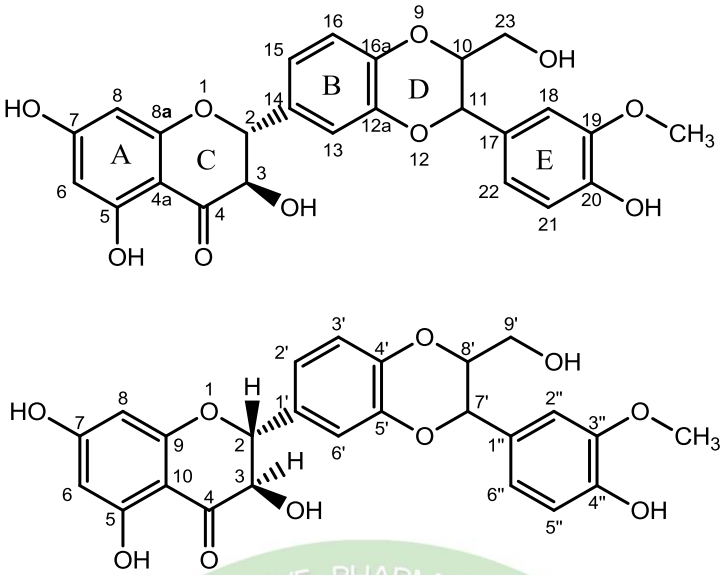

Fig.4: Structure of Silymarin

STEREOCHEMISTRY OF FLAVONOLIGNANS

There are ^{1}H and ^{13}C NMR similar spectra for Silybin A, Silybin B, Isosilybin A and Isosilybin B and no distinctive signals for easy isomer verification. The reverse phase HPLC showed significant differences in retention times for these isomers.Sequential silica gel column chromatography separated these diastereoisomeric pairs, HPLC preparative reversed phase, and CH_2Cl_2-MeOH recrystallization [34]. which allowed isolation of pure diastereoisomers. ^{1}H NMR spectrum from the observed coupling constants (11.4 and 8.1 Hz) confirmed the trans formation ofH-2,H-3 and L-H-7 L-H-8 in Silybin A. Stereochemistry was determined by silybin dehydrogenation at 2R and 3R[43]. The stereochemistry of C-7′and C-8′ was constructed as 7′R and 8′R by comparison of the optical rotation withthat of isosilybin A, which was determined by X-ray crystallography. The ^{1}H NMR spectrum of isosilybin B is very similar to that of silybin A with differences of less than 0.01 ppm in chemical shifts.The 1H NMR spectrum of a silybin A and silybin B mixture is similar to that of a single compound. The difference in chemical shifts in silybin A and silybin B spectra of ^{13}C NMR is not greater than 0.06 ppm. The 7′S and 8′S configurations were assigned based on the differences in optical rotation between silybin A ($[\alpha]_D$+2.0°)and silybin B ($[\alpha]_D$-1.07°). The stereochemistry of isosilybin A was assigned by X-ray crystallography in combination with optical rotation data as 2R, 3R, 7′R, and 8′R. The significant differences in optical rotation between isosilybin A ($[\alpha]_D$+48.15°) and isosilybin B ($[\alpha]_D$-23.55°)confirmed the configuration70S and 80S. Isosilybin C and isosilybin D have been isolated as small compounds from the milk thistle extract.The regiochemistry of both these compounds is similar to isosilybin A and isosilybinB.The major structural difference between these

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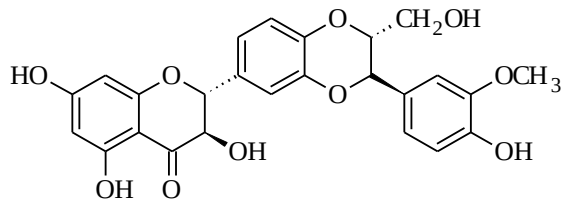
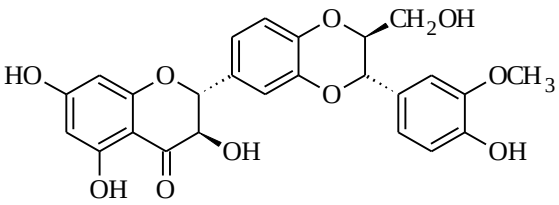
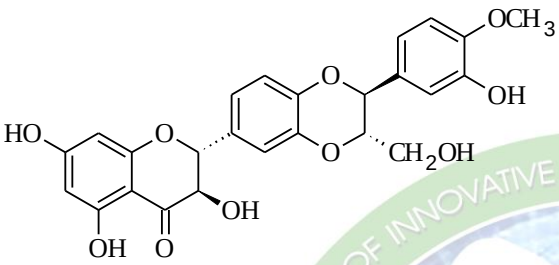
compounds and other flavonolignans is 1,3,5 substitution pattern in the aromatic ring derived from lignin [42,44].



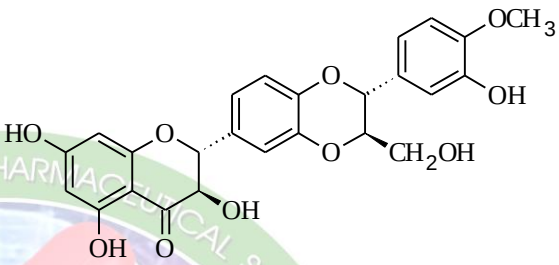
Silybin A



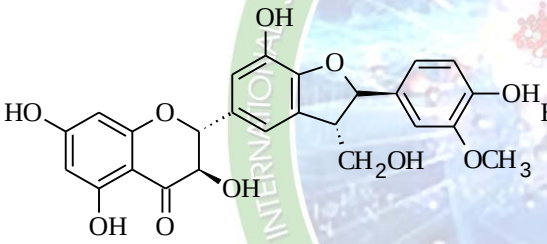
Silybin B



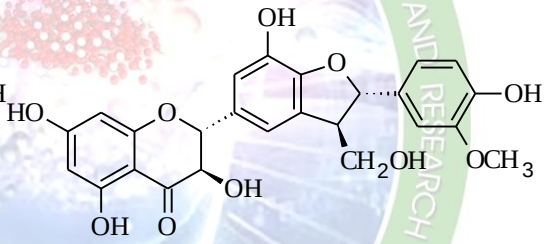
Isosilybin A



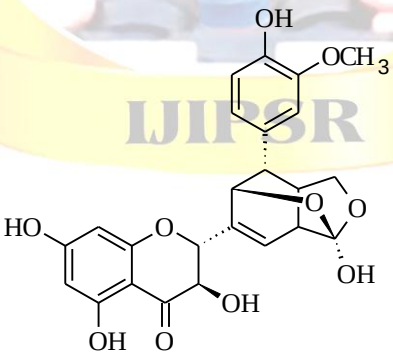
Isosilybin B



Silychristin A



Silychristin B



Silydianin

Fig.5: The primary Structures of flavonolignans from milk thistle.

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PHARMACOLOGY

Most biochemical and pharmacological studies with a standardized silymarin preparation or its main component, silybin, have been carried out.

ANTIOXIDANT ACTIVITY

T. Parasassi *et.al.*, extracted Silymarin and silibyn from the *Silybum marianum* seeds and used by their free radical scavenger as a protective liver. When embedded in rabbit liver microsomes, the anisotropic fluorescence of 1,6-diphenyl-l,3,5-hexatrien(DPH), but not 1-anilinonaphthalene-8-sulphonic acid(ANS), which is incorporated into the membranes, decreased slightly. However, they reduce the fluorescence intensity of the included ANS without altering the maximum wavelength. These observations suggest that the drugs are part of the microsomal interface of the hydrophobic-hydrophilic bilayer and interfere with the structure by affecting the packingof the acyl chain. After 45 minutes of incubation, almost complete labeling of the liver microsomes can be obtained either by using DPH or ANS, which was the time used to label microsomes. DPH anisotropic values of liver microsomes depending on the silymarin or silybin concentration. The microsome concentrations for all drug concentrations (corresponding to 0.2 mM phospholipids) were kept constant. A small decrease in DPH anisotropic values indicates an increased freedom of rotation of the sample in the presence of silymarin and silybin at a molar ratio of 0.5 drugs to phospholipids. Anisotropic values are practically constant for higher concentrations of drugs. Similar ANS experiments show no variation in fluorescence anisotropy at any concentration of drugs. However, the ANS fluorescence intensity in labelled microsomes decreases depending on the concentration of the drug without shifting the maximum intensity. No variations in the activity of 5'-nucleotidase were observed in the range of drug concentrations studied [45].

Cavallini, L. *et. al.*,compared the antiperoxidative effect of silymarin with quercetin, dihydroquercetin and quercitrine: dihydroxyfumaric acid plus $FeSo_4$ and potassium peroxychromate, in rat liver microsomes and mitochondria which was exposed*in vitro* to two peroxidation systems, Silymarin has been found and showed the same activity as quercetin and dihydroquercetin and as antiperoxidant is more active than quercitrin, regardless of the peroxidation system. The results also shows that all the tested flavonoids act as free radicals and not just as metal complexing agents [46].

M.L. Mira. *et. al.*, had showed that 3 compounds, silibin, sorbinil and bendazac, have *in vitro* antioxidant properties, as scavengers of Fenton-generated hydroxyl radical. Silibin proved to be the strongest scavenger and appears to have iron binding properties, as in the presence and absence of EDTA its activity is the same. The fact that these compounds have antioxidant

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properties can help to explain the effect of bendazac and sorbinil on cataract and silibin as a protector of liver parenchyma. However, it should also be remembered that although a drug is an active OH· scavenger in simplified *in vitro* systems, other factors can interfere *in vivo* with its effects. However, these factors include the toxicity, short biological systems half-life and non-ideal distribution of tissue. To be effective in a biological system, the scavenger must be in the right place, at an appropriate concentration, and both the scavenger and its reaction product must be toxic [47].

A. Bindoli, *et. al.*, reported that there was unambiguous evidence that Silymarin had a remarkable antioxidant effect on both liver mitochondria and microsme when exposed to peroxidative systems. The antioxidant action of Silymarin in liver mitochondria is demonstrated by the concomitant inhibition of Fe^{2+} swelling caused by ascorbate. Oxygen absorption and MDA formation and all these parameters were an expression for the formation of lipid peroxides. Silymarin was able to prevent peroxide formation and inhibit it when added to the system after the process had begun. As is well known, peroxidant-induced mitochondrial swelling reflects irreversible changes in the membrane structure due to lipid peroxidation. Since Silymarin inhibits lipid peroxidation (oxygen absorption and formation of MDA) to the same extent as it inhibits the swelling of mitochondria, it seems reasonable to assume that Silymarin 's " stabilizing " action is the result of its antioxidant effect. In addition, the antioxidant action of Silymarin was about ten times higher than the equimolar concentration of α-tocopherol. The potential consequence of the antioxidant capacity of flavonoids is the chelate of metal ions, in particular to Fe^{2+} or Cu^{2+}.Although chelation of added or endogenous Fe cannot be excluded for Silymarin in absolute terms, the results indicate that this option is unlikely. In fact, even after the pre- treated mitochondria was repeatedly washed, the antiperoxidative action of Silymarin persisted and was then brought into contact with amounts of Fe^{2+} well above the original Silymarin concentration. It seems very unlikely that residual Sliymarin can successively chelate all the Fe^{2+} ions added to the system [48].

C. F. Manso*et. al.*,had assessed the antioxidant properties of SDH by examining the drug's capability to respond with appropriate biological oxidants such as radical superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$) and hypochlorous acid (HOCl).It has also been investigated for its effect on lipid peroxidation. SDH was not a good O_2^-scavenger and no H_2O_2 reaction has been detected within their test sensitivity limit.Indeed, it interacts quickly with HO radicals in free solution at a diffusion-controlled rate (K= (1.0-1.2) X l010/M / sec) and appears to be a weak ion chelator. At concentrations in the micromolar range SDH

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protected against HOCl inactivation a1-antiproteinase, showing that it is a powerful scavenger of this oxidizing species. Luminol-dependent HOC1-induced chemiluminescence was also inhibited by SDH. The reaction of SDH to HOC1 was monitored by the modification of the SDH UV-visible spectrum.Studies with rat liver microsome lipid peroxidation induced by Fe(III)/ascorbate have shown that SDH has an inhibitory effect that depends on its concentration and lipid peroxidation magnitude. This work wants to support the reactive action of the SDH-attributed scavenger oxygen species [49].

Valenzuela *et. al.,* observed that a differential effect of silybin dihemisuccinate on rat liver microsonal oxygen consumption and NADPH-Fe^{2+}-ADP and t-butyl hydroperoxide lipid peroxidation. These results are attributed to flavonoid's antioxidant properties. The discrepancies in the effect of the catalysts may be due to the different silybin capacity to act as a scavenger of free radicals formed by NADPH-Fe^{2+}-ADP or t-butyl hydroperoxide [50].

H. Farghaliu *et. al.,*were investigating the potentials of silymarin for hepatic injury betterment. The possible mechanism that contributes to silymarin 's hepatoprotective effect and the role of intracellular calcium (Ca_i^{2+}), which a model of isolated and immobilized hepatocytes with tert-butyl hydroperoxide (TBH) and D-galactosamine (D-Gal) intoxication was investigated.Silymarin reduced leakage of lactate dehydrogenase (LDH), increased oxygen uptake, decreased lipid peroxide formation (malondialdehyde, MDA) in TBH-altered hepatocytes and increased urea synthesis in the perfusion medium.TBH treatment significantly increased Ca_i^{2+} in hepatocytes to over 600 nM and silymarin pre-treatment reduced the TBH-induced increase in $Ca_i^{2+}$and reduced $Ca_i^{2+}$to below 300 nM. Silymarin did not affect LD leakage or urea synthesis in D-Gal-injured cells.It follows that under the present test conditions the hepatoprotective effect of silymarin is caused by the inhibition of lipid peroxidation and that hepatocyte modulation Ca_i^{2+} plays a key role in a protective action [51].

ANTI-INFLAMMATORY ACTIVITY

Miadonna A. *et. al.,* were examined the effect of a naturally produced flavonoid, silybin, on the release of histamine from human basophils to assess the potential utility of allergic disorders.The release of f-met peptide and anti-IgE-induced histamine was significant ($P < 0.05$)and was concentration-dependent inhibitor. In contrast, no significant ($P > 0.05$) effect on calcium ionophore A23187 has been documented.The inhibitory activity ($P < 0.05$) was significantly reversed by increasing extracellular calcium concentrations.The anti-allergic properties of silybin can reasonably be attributed to membrane-stabilizing activity, possibly in connection with calcium influx interference.These results suggest that an *in vivo* evaluation of silyhin 's anti-allergic activity would be meaningful [52].

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R. Fantozzi. *et. al.,* was found that human neutrophils, activated by N-formylmethionyl-leucyl- phenylalanine(FMLP) chemotactic peptide, evoke the release of histamine from serosal mast cells of rats.The release depends on the FMLP concentration and can be inhibited by disodium cromoglycate and flavonoid silymarin, which has free radical scavenging characteristics.silymarin is caused inhibition of release of neurtophil-mediated histamine is a dose dependent process.These results further emphasize the idea of a neutrophil-mast cell interaction that can be involved in inflammatory processes [53].

F. Fiebrich *et. al.,* was examined that *in vitro* studies reveals that the main phytoconstituents of silymarin, have an inhibitory effect on prostaglandins formation, they are Silybin, silydianin and silychristin. The effectors concentration has log-linearly which shows the effect of inhibition. Among other things, the antihepatotoxic effect of silymarin is due to a special " protective effect " that the active components of this natural substance have on certain membrane lipoids. In the previous report, they showed that these substances inhibit lipoxygenase activity in a specific way. However, Silybin almost inhibited the formation of PG at the highest concentration (0.3 mM), in the most extreme case, silydianin and silychristin reduced it to about a third or a half of the unchecked PG production respectively. Silybinwas thus the strongest inhibitor of *in vitro* PG synthesis. In the case of partial destruction of membrane structures in the liver following organic diseases or intoxications, increased amounts of C20 fatty acids are released through lipolysis, which leads, amongst other things, to increased PG synthetase activity. Silymarin can counteract this harmful process. The suppression of the (pathological) decomposition of membrane lipoids together with the inhibition of PG formation could provide a reasonable explanation for the functioning of this hepatotropic complex [54].

R. De La Puerta.*et. al.,* tested *in vivo* anti-inflammatory activity of silymarin for acute inflammation in different experimental models. Silymarin given orally in carrageenan-induced paw oedema in rats that reduced dose-dependent food-pad abscesses (ED50=62,42 mg kg-1).In xylene-induced inflammation of the ear mouse, silymarin was applied topically more efficiently than intraperitoneally, with effects comparable to indomethacin. Silymarin also generated a dose-dependent inhibition of leukocyte aggregation in inflammatory exudates after carrageenan injection in mice; silymarin reduced the number of neutrophils significantly. In the *in vitro* test, Silymarin could not inhibit phospholipase A2.These results suggest that silymarin has a significant *in vivo* anti- inflammatory effect by reducing oedema with a marked effect of neutrophil migration inhibition to the inflammed site [55].

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C. Alarcon de la Lastra,*et. al.,* found that the oral silymarin treatment in rats to be effective for preventing gastric ulceration caused by cold-restraint stress. Statistically significant values of the ulcer index were observed for the control group. Silymarin showed significant reduction in the number and 70 ulcers in 6 h pyloric animals, although the concentration of histamine was significantly reduced, it did not alter the volume or acidity of gastric secretion. Treatment with silymarin for 1 or 2 hours prior to the antiulcerogenic agent in absolute ethanol-induced ulcers did not prevent the formation of gastric lesions. In addition, the hexosamine content was significantly reduced, but the overall protein output was increased, showing similar values to the standard drug carbenoxolone. These results indicate that the anti-ulcerogenic effect of silymarin could be directly linked to its inhibitory proxidation mechanism by the lipoxygenase pathway, avoiding leukotriene synthesis [56].

HEPATOPROTECTIVEACTIVITY

Julio C. Davila *et. al.,* used primary neonatal hepatocyte cell cultures to examine the protective effect of hepatotoxins on flavonoids. Catechin (CAT) and silybin (SIL) protected hepatocytes from cell injury resulting from erythromycin estolate (EE), amitriptyline (AT), nortriptyline (NT) and tert- butylhydroperoxide (TBOOH).As indices of hepatotoxicity were the leakage of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as morphological parameters. Hepatocytes have been exposed to EE(1×10^{-4} M and 2×10^{-4} M), AT, NT and TBOOH for a 2-hour period. In comparison with untreated control groups, these hepatotoxins caused significant leakage of LDH, AST and ALT ($P < 0.05$).NT has been less toxic than its parent, AT. After 1 hour of treatment with toxicants, changes in morphology were evident, including: vacuol formation, deformation in size and cell necrosis. As the hepatotoxin concentration increased, the changes were more marked. Pretreatment of cultures with either CAT or SIL led to less enzyme leakage and hepatotoxin morphological changes. The results of this study indicate that CAT and SIL can be used to stabilize the plasma membrane against toxic insults [57].

Hiroshi Hikino *et. al.*,examined the antihepatatoxic effects of flavonolignans and related components of *Silybum marianum* using carbon tetrachloride and galactosamine- induced cytototoxicity as model systems in primary rat hepatocytes.Significant inhibitory actions were observed with silandrin,3-deoxysylchristin, silybin and silymonm in carbon tetrachloride-treated cultures, and silydianin and silymonin effectively prevented cell lesions produced by galactosamine [58].

Kai O. Lindros *et. al.,* investigated alcohol-inducing cytochrome P450 2E1 which is involved in the hepatoprotective mechanism of the plant flavonoid extract silymarin and its main active

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component silybin in isolated hepatocytes.Allyl alcohol toxicity, lipid peroxidation and GSH depletion were effectively counteracted by silymarin (0.01- 0.5 mM) and silybin at higher concentrations.Cell damage from t-butyl hydroperoxide has also been prevented, but less efficiently, by silymarin and silybin.However, the addition of flavonoids did not affect the covalent binding of intermediate acetaminophen formed through P450 2E1.Silybin did not affect N-nitrosodimethylamine microsomal 2E1-catalyzed demethylation.Neither did N-nitrosodimethylamine nor aminopyrine demethylation by isolated microsomes affects*in vivo* silybin administration. Addition of silymarin or silybin to primary cultures of isolated hepatocytes did not prevent cell damage induced by exposure to the P450 2E1 substrate CCl_4. In contrast, the mere presence of low concentrations (25- 50 μM) of these compounds has been found to inhibit cell attachment to the matrix and ultimately cause cell damage.They conclude that, contrary to previous reports, there was no evidence of silymarin or silybin interactions with cytochrome P450 2E1.This suggests that the antioxidant and free radical scavenging properties of these compounds can account for the majority of the therapeutic effect [59].

FaustoMachicao *et.al.,* investigated the influence of flavonolignan derivatives silybin, silybinhemisuccinate and silybindihemisuccinate on RNA synthesis in isolated nuclei of rat livers.A stimulating effect of silybin on the incorporation of [^{3}H] UTP into the nuclear RNA has been identified and dose dependence has been shown.The results support previous findings that silybin stimulates *in vivo* rat livers and hepatocytes the synthesis of RNAs. Experiments with purified nuclei showed that the synthesis rate of rRNA is mainly increased. The stimulation mechanism was clarified by isolated RNA polymerases experiments. Polymerase B may not be affected by flavonolignane, but polymerase A activity is increased at constant Km values due to higher V.Silybin therefore proves to be an enzyme stimulating agent [60].

Sonnenbichler J. *et. al.,* demonstrated that Silibinine increases the synthetic rate of RNA ribosomal species 5.8s, 18s and 28s by about 20 percent not only in rat livers and hepatocyte cultures, but also in isolated liver nuclei through activation of DNA- dependent polymerase I. The formation of mature ribosomes is then stimulated and a significant consequence of the protein biosynthesis in the livers is also increased.The mitotic activity of a cell is regulated by proliferation active factors in conjunction with RNA and protein synthesis. Therefore, with regard to pharmacological aspects, it was important to investigate any influence of Silibinin on DNA replication. They describe Silibinin 's influence on DNA synthesis in normal rat livers and in partially hepatectomized rats in livers. They used rat and human hepatoma cells and

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HeLa-and Burkitt lymphoma cell cultures for rapidly growing cells. In hepatectomized livers, they observed a remarkable increase in DNA synthesis due to the flavonolignan derivative. There was no effect in normal livers or malignant cell lines [61].

M. Mourelle *et. al.*, studied the efficacy of silymarin treatment to prevent biochemical and histological changes in the liver cirrhosis caused by CC_{l4} in rats. Four groups of rats have been treated with:(1) CC_{l4};(2) mineral oil;(3)CC_{l4}+silymarin; and (4) silymarin. 72 hours after the treatments were completed, all animals were sacrificed.The serum tested determined the activity of alkaline phosphatase (alk. phosp.), gamma-glutamyl transpeptidase (GGTP), glutamic pyruvic transaminase (GPT) and glucose-6-phosphatase (GPase) and bilirubin content. The activity of Na^+, K^+-ATPase and Ca^{2+}-ATPase in isolated plasma membranes has been measured. In liver homogenates also lipoperoxidation, triglycerides (TG) and glycogen content have been measured. Liver cirrhosis was shown by substantial increases in liver collagen, lipoperoxidation, alkaline serum activity. GGTP, GPT, G6Pase. Phosp. Content of bilirubin and TG liver.The activity of ATPases in plasma membranes was significantly reduced, as was the content of liver glycogen.All changes in CC_{l4}-cirrhotic rats were completely prevented, with the exception of liver collagen content, which was reduced by only 30% compared to CC_{14}-cirrhotic rats co treated with Silymarin (50 mg / kg b.wt).The result suggests that antioxidant and membrane stabilizing actions of the agent can be likened to Silymarin protection [62].

Pablo Muriel *et. al.*, were studied activities of Ca^{2+} and Na^+, K^+-ATPases in CC_{l4}-cirrhotic rat liver plasma membranes and rat livers treated with silymarin in addition to CC14.CCl4 chronic therapy has led to significant decreases in Na^+, K^+-and Ca^{2+}-ATPase;However, the animals treated with silymarin and CC_{l4} did not show any differences in the activity of ATPase compared to controls. The lipid analysis in plasma membranes showed increases in the cirrhotic group, in cholesterol / phospholipid (CH / PL) and sphingomyelin / phosphatidylcholine (SM / PC) ratios. Again, the membranes isolated from CC_{l4} + silymarin rats showed normal values of CH / PL and SM / PC, Since the CH / PL and SM / PC ratios are related to the microviscosity of the membrane. This study shows that lower membrane fluidity in the cirrhotic group can be responsible for the observed decreases in ATPase activity. In addition, the role of silymarin in improving liver function in CC_{l4}-cirrhosis can be attributed partially to its membrane-level action by preventing increases in CH / PL and SM / PC ratios [63].

Meiss R, *et. al.*, studied the Silybin effect on the hepatic cell membranes following polycyclic aromatic hydrocarbon (PAH) damage.In a suspension of 0.5 ml tricapryline to young female

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NMRI mice, a single intraperitoneal injection of a PAH mixture containing 3 micrometers of benzo(a)pyrene was given. Three and four days later, a number of mice received Silybin injections of 100 mg / kg of body weight in tail veins. Thin sections of the animal's livers have been investigated using the freeze fracture technique under the electron microscope. In addition to marked changes in the hepatocytic nuclei and mitochondria, marked dilatation of intercellular space and frequent occurrence of intracytoplasmic vacuoles was observed following intraperitoneal PAH suspension injection and without Silybin treatment. These changes were no longer observed after administration of Silybin, although 50 percent of the cell membranes showed a marked band-like proliferation of the tight junctions. This observation can be interpreted to support the postulated membrane-stabilizing effects of Silybin [64].

Floersheim, G. L. *et. al*., studied the Penicillin and Silymarin effects on liver enzymes and blood coagulation factors in dogs based on boiled *Amanita Phalloides* preparation. Sublethal doses of Amanita phalloides (death cap) were given to dogs, and serum enzymes and coagulation factors were monitored for liver injury, a major feature of this intoxication. The tested antidotes were selected from a variety of agents that had some death cap toxin antagonism in previous experiments with rats and mice.This experiment suggested that penicillin G and silymarin prevented the growth of liver enzymes in the blood of poisoned dogs and reduced coagulants. Cytochromium C, prednisolone and cimetidine were inefficient [65].

P. Ferenci *et. al.,* was studied the randomized controlled silymarin treatment trial in patients with liver cirrhosis. In 170 cirrhosis patients, a double-blind, prospective, randomized study was conducted to determine the effect of silymarin on cirrhosis outcomes.87 patients received 140 mg silymarin three times daily (alcoholic 46, non-alcoholic41; 61 males, 26 females; child A, 47; B, 37; C, 3; average age 57).Placebo was given to 83 patients (alcoholic 45, non-alcoholic 38; 62 males, 21 females; children A, 42; B, 32; C, 9: average age 58).Non-compliant patients and patients unable to be controlled were considered " withdrawals " and withdrawn from the study. All patients received the same treatment until the last patient was treated for 2 years. The average observation time was 41 months. There were 10 and 14 dropouts in the placebo and treatment group respectively. In the placebo group, 37 patients (+ 2 dropouts) died and 31 of them died from liver disease.24 (+ 4 dropouts) had died in the treatment group and 18 of these deaths were related to hepatic disease. The 4-year survival rate for silymarin- treated patients was 58 ± 9 per cent (S.E.) and 39 ± 9 per cent for placebo (P= 0.036).Subgroup analysis showed that treatment was effective in patients with alcoholic

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cirrhosis (P=0.01) and initially rated Child A' (P=0.03) in patients.No drug treatment side effects were observed. The results of this study indicate that the mortality of cirrhosis patients has been reduced by silymarin treatment [66].

ANTI-DIABETIC ACTIVITY

Bruno Guigas *et. al.*, studied Silibinin effects on the gluconeogenesis of the liver by titrating starving rat hepatocytes with sub-saturating concentrations in a perifusion system of various exogenous substrates. The endogenous glycogenolysis was also investigated by hepatocytes from fed rats. The effect of Silibinin on glucose-6-phosphatase kinetics in intact and permeable microsomes of the rat liver was determined. The results were found to induce dose-dependent gluconeogenesis inhibition associated with a significant reduction in glucose-6-phosphate hydrolysis.All gluconeogenic substrates, i.e. dihydroxyacetone, lactate / pyruvate, glycerol and fructose, have been shown to be effective. Morever, silibinin reduced both gluconeogenesis and glycogenolysis stimulation associated with reduction glucose-6-phosphate hydrolysis.glucose-6-phosphatase in rat liver microsomes inhibited by silibinin in a concentration-dependent manner which shows the decrease in intact cell Glucose-6-phosphate hydrolysis. This experiment suggests that silininin shows inhibitory effect on hepatic glucose-6-phosphatase and gluconeogenesis which may be interesting in type 2 diabetes mellitus therapy [67].

Mohamad-Yehia El-Mir *et. al.*, examined the effect of silibinin on both the oxidative use of glucose and the reactive oxygen generation (ROS).Since the secondary release of ROS to an increased mitochondrial metabolism can lead to diabetic damage. They found that carbohydrate glycolysis depended on silibinin dose in a cell perifusion system due to an inhibitory effect on pyruvate kinase activity. In addition, there has been a dramatic effect on oxidative phosphorylation, as shown by a reduction in the ATP-to-ADP ratio and an increase in the lactate-pyruvate ratio. The most desirable finding was that silibinin completely mitigated the increase in ROS formation driven by metabolic flow at a concentration of as little as 10 μM.The low dose of silibinin reduced the production of ROS in isolated liver mitochondria due to the activity of the electron transfer chain.These results suggest that interesting silibinin activities could be expected from its potential clinical use, particularly in pathological conditions where the formation of mitochondrial ROS is significantly improved [68].

Claudia P. Soto *et. al.*,studied the results of silymarin on rats pancreatic, hepatic and blood glutathione (GSH), collectively with pancreatic concentrations of alloxane malondialdehyde. Silymarin alone increases the pancreatic and blood GSH without altering glucose levels in the

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hepatic or blood. Increase of lipid peroxidation by means of alloxane prevented with silymarin. It also blunts the continuous increase in alloxan-induced plasma glucose.It was suggested that silymarin protect the pancreatic damage of experimental diabetes mellitus.This may be related to its antioxidant properties and the increase in plasma and pancreatic glutathione concentrations [69].

They also aimed to study and test the silymarin in two protocol to investigate that silymarin could lower hyperglycemia and reverse pancreatic damage in rats treated with alloxan. The consequences showed that serum glucose increased and serum insulin declined significantly 72 hours after management of alloxan.

While morphological abnormalities like islet shrinkage, necrotic areas, cell loss, widespread lipoid deposits in the exocrine tissue and loss of beta cells were present in pancreatic tissue, insulin and glucagon immunoreactivity were scattered if any.In comparison, insulin and glucose serum levels of silymarin-treated rats were similar to those of control animals.In addition, immunocellular patterns of insulin and glucagon in Langerhans islets were also normal, and normal patterns of insulin and Pdx1 mRNA expression were detected during pancreatic recovery in Langerhans islets. The results suggest that silymarin induces pancreatic function recovery confirmed by insulin and glucagon expression protein and normoglycemia after alloxan pancreatic damage in rats [70].

Hao-Ming Tian*et. al.*,studied the possible effects of silybin and its underlying mechanisms on experimental diabetic retinopathy and investigated them. Diabetes was induced with streptozotocin (STZ) plus a high fat diet in Sprague-Dawley rats and 22 weeks after diabetes was induced, silybin was administrated. In order to evaluate the intercellular adhesion molecule-1 (ICAM-1) of the retinal capillaries, leukostasis and levelofretinal, histochemical and immunofluorescence techniques have been used. Western blot was implemented to quantitate the retinal expression ICAM-1.Results show that silybin therapy significantly prevented the development of obliterated retinal capillaries in diabetes compared to the treatment of vehicles.In addition, leukostasis and retinal ICAM-1 levels have been found to decrease significantly in diabetic groups treated with silybin.The results indicate that silybin reduces obliterated retinal capillaries in experimental diabetes and that the recovered vascular leukostasis of the retinal leukostasis and ICAM-1 levels contribute to the preventive effect of silybin at least partially [71].

Sun-o Ka*et. al.*, investigated the effects of silibinin on adipogenesis. Treatment with silibinin suppressed terminal differentiation of 3T3-L1 cells into adipocytes as demonstrated by staining the results of the Oil Red O and TG test. Real-time analysis of RTPCR revealed that

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silibinin reduced the expression of adipogenesis-related genes such as CAAT / enhancer binding protein-α, fatty acid synthase, protein1c binding sterol response element, adipocyte-specific lipid binding protein, peroxisomeproliferator-activated receptor Á and lipoprotein lipase, and increased the expression of preadipocyte factor-1, a preadipocyte marker gene. The anti-adipogenic effect of silibinin was associated with the up-regulation of insig-1 and insig-2. Collectively, these results suggest that silibinin inhibits adipocyte differentiation through a potential upregulation of insig-1 and insig-2 at an early phase in adipocyte differentiation [72].

M. Velussi *et. al.*, subjected patients with diabetes caused by alcoholic liver cirrhosis, 60 patients (42 men), aged 45 to 70 years. Each patient received insulin and showed high endogenous insulin secretion. Patients were randomly assigned 600 mg of silymarine daily or no silymarine for 6 months. The mean levels of fasting blood glucose, daily blood glucose, daily glycosuria, glycosylated hemoglobin, daily insulin need, fasting insulinemia, blood malondialdehyde and basal and glucagon-stimulated C peptide in silymarin-treated patients were all considerably lower than in untreated patients and lower than in baseline. The results suggest that silymarin can reduce lipoperoxidation of liver cell membranes in cirrhotic diabetic patients and reduce the endogenous secration of insulin and the need for exogenous insulin, possibly by restoring the liver cell plasma membrane and increasing insulin receptor sensitivity [73].

Jorn Dietzmann *et. al.*, investigated the relationship between the cellular thiol status and T-cell activation and cytokine synthesis of mononuclear cells in patients undergoing dialysis treatment with end-stage diabetic nephropathy (ESDN). The thiol status of the peripheral blood lymphocytes of 30 patients with ESDN hemodialysis and healthy controls was determined by flow cytometry.CD69 expression has been used to analyse T-cell activation in response to pokeweed mitogen; cytokines have been determined in supernatant cell culture. As a result, a significant thiol deficiency in patients with ESDN was evident in comparison to age-related healthy subjects. The lower intracellular thiol levels directly correlated with a significant decrease in T-cell activation and a higher TNF-α synthesis in the patient group. Flavonoid treatment has resulted in the restoration of thiol status *in vitro* and *in vivo* within 72 hours. This effect indicated a biphasic kinetic which first used thiols on the cell surface and secondly intracellular thiols. In direct comparison, the activation of T-cells was significantly improved and the release of TNF-alpha was substantially reduced.These data rationalize the normalization of immunoregulatory defects in clinical trials using flavonoids in ESDN through the restoration of cell thiol status [74].

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Banafshe Dormanesh *et.al.,* studied 60 patients with macroalbuminuria type 2 diabetes(urinary albumin excretion >300 mg/24 h). Contrary to medication with the maximum dose of the inhibitor of the renin-angiotensin system for more than 6 months and with an estimated glomerular filtration rate of 30 mL / min/1,73 m^2.For 3 months, patients were randomly selected to two equal groups to receive three 140 mg tablets of silymarin or three placebo tablets daily.The primary result was an absolute change in the urinary albumin-creatinine ratio (UACR) from start to finish of the treatment phase. UACR and TNF-α urinary and serum levels (tumour necrosis factorα; inflammatory marker), malondialdehyde (MDA; oxidative stress marker) and TGFβ (transformation factorβ; fibrosis marker) at baseline and at the end of the treatment phase. Although UACR decreased in both groups, this decrease in silymarin was significantly higher than in placebo; the mean difference in UACR change between the two groups was-347 (95 percent Cl-690 to-4) mg / g. Urinary TNF-α and urinary and serum MDA levels also decreased substantially in silymarin compared to placebo. They suggest and concluded that Silymarin reduces urinary excretion of albumin, TNF-α and MDA in diabetic nephropathy patients and can be considered a novel addition to the anti-diabetic nephropathy armamentarium [75].

H. Fallah Huseini *et. al.,* studied and designed an experiment to explore the effects of herbal medicine, *Silybum marianum* seed extract (silymarin), which in diabetic patients is known to have antioxidant properties on the glycemic pattern. A randomized four-month double-blind clinical trial was conducted in 51 patients with diabetic type II in two well-adjusted groups. The first group (n=25) received a 3 times daily silymarin tablet (200 mg) plus conventional therapy. The second group (n=26) received the same therapy, but instead of silymarin a placebo tablet. The patients were checked monthly and glycosylated haemoglobin (HbA1c), fasting blood glucose (FBS), insulin, total cholesterol, LDL and HDL, triglyceride, SGOT and SGPT levels were determined at the beginning and the end of the study. The results showed a significant decrease in HbA1c, FBS, total cholesterol, LDL, triglyceride SGOT and SGPT levels in patients treated with silymarin compared with placebo and with values at the start of the study in each group. Finally, silymarin treatment in type II diabetic patients has a beneficial effect on the improvement of the glycemic profile for 4 months[76].

CONCLUSION

It has been shown that flavonolignans reveal extensive range of therapeutic activity including antioxidant, anti-inflammatory, hepatoprotective, antidiabetic properties. In this review, selected vernacular names, history, chemistry, selected pharmacological action of silymarin and various techniques were studied to analyse the content and composition of the active

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principle in silymarin plant material expelled. We conclude that Synthetically and semisynthetically, nucleus and peripheral modifications, either in the form of skeletal rearrangements or partial degradations as well as functional group addition and replacement giving access to new structural motifs, which can be used in future as a lead compounds.

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