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Dedication

This thesis is dedicated to our beloved parents, whose unwavering love, sacrifices, and constant encouragement have been the foundation of our academic journey. Their faith in the power of education and their enduring support have been a guiding light throughout every challenge and achievement.

We also wish to express our heartfelt gratitude to our siblings, whose presence, understanding, and quiet strength have provided us with comfort and motivation during our most trying moments.

Our sincere appreciation goes to our esteemed professors and academic mentors, whose insightful guidance and continuous support have significantly shaped the quality and depth of this work.

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Mm.Berber Naima, for the honor you do us by agreeing to examine this work in order to evaluate it.

Finally, we express our gratitude to all those who helped us directly or from afar.

List of Abbreviations

- **aP2:** Adipocyte Protein 2
- **Akt:** Protein Kinase B
- **ALT:** Alanine Aminotransferase
- **AMPK:** AMP-Activated Protein Kinase
- **AST:** Aspartate Aminotransferase
- **CAMs:** Complementary and Alternative Medicines
- **CAT:** Catalase
- **C/EBPα:** CCAAT/Enhancer Binding Protein Alpha
- **ERK1/2:** Extracellular Signal-Regulated Kinases 1 and 2
- **FAS:** Fatty Acid Synthase
- **G6Pase:** Glucose-6-Phosphatase
- **GLUT4:** Glucose Transporter Type 4
- **GPx:** Glutathione Peroxidase
- **GSH:** Reduced Glutathione
- **HbA1c:** Hemoglobin A1c
- **hIAPP:** Human Islet Amyloid Polypeptide
- **HOMA-IR:** Homeostatic Model Assessment for Insulin Resistance
- **IL-1β:** Interleukin-1 beta
- **IL-6:** Interleukin-6
- **iNOS:** Inducible Nitric Oxide Synthase
- **LPL:** Lipoprotein Lipase
- **MAPK:** Mitogen-Activated Protein Kinase
- **NAFLD:** Non-Alcoholic Fatty Liver Disease
- **NASH:** Non-Alcoholic Steatohepatitis
- **NF-κB:** Nuclear Factor-kappa B
- **NO:** Nitric Oxide
- **PEPCK:** Phosphoenolpyruvate Carboxykinase
- **PI3K:** Phosphoinositide 3-Kinase
- **PPARγ:** Peroxisome Proliferator-Activated Receptor Gamma

- **ROS:** Reactive Oxygen Species
- **SOD:** Superoxide Dismutase
- **SREBP1c:** Sterol Regulatory Element-Binding Protein 1c
- **STZ:** Streptozotocin
- **T2DM:** Type 2 Diabetes Mellitus
- **TNF-α:** Tumor Necrosis Factor-alpha

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Abstract

Diabetes mellitus is a chronic metabolic disorder marked by persistent hyperglycemia due to impaired insulin secretion, insulin resistance, or both. Given the rising global prevalence of diabetes, there is growing interest in complementary therapies alongside conventional treatments. This thesis investigates the therapeutic potential of *Silybum marianum* (milk thistle), focusing on its bioactive constituent silymarin, through an integrative approach combining literature review, mechanistic evaluation, and field-based survey data.

The pharmacological review highlights silymarin's antioxidant, anti-inflammatory, and insulin-sensitizing properties, which contribute to improved glycemic control. A quantitative survey involving 100 diabetic patients and 10 healthcare providers revealed that although 76% of patients were aware of milk thistle, only 3% had used it for diabetes management. Among those users, all reported reductions in fasting blood glucose without adverse effects, despite not consulting their physician. Additionally, 79% of patients indicated willingness to use milk thistle if medically endorsed. Among physicians, 50% were aware of its potential benefits, though none had recommended it due to insufficient clinical evidence. Nevertheless, 70% viewed it as conditionally safe and expressed openness to future use pending stronger scientific validation.

In conclusion, while silymarin demonstrates promising antidiabetic mechanisms, its integration into clinical practice requires robust clinical trials, regulatory oversight, and physician education to ensure evidence-based application in diabetes care.

Keywords: Diabetes mellitus, Milk thistle (Silybum marianum), Silymarin, Insulin resistance, Oxidative stress, Glycemic control.

الملخص

يُعدّ داء السكري اضطرابًا استقلابيًا مزمنًا يتميز بارتفاع مستمر في نسبة السكر في الدم نتيجة ضعف إفراز الإنسولين أو مقاومة الإنسولين أو كليهما. ومع تزايد انتشار السكري عالميًا، يزداد الاهتمام بالعلاجات التكميلية إلى جانب العلاجات التقليدية. تبحث هذه الأطروحة في الإمكانيات العلاجية لنبات الحرشف البري *Silybum* (*marianum*مع التركيز على مركبه النشط السليمارين، من خلال منهج تكاملي يجمع بين مراجعة الأدبيات والتقييم الآلي وبيانات الاستقصاء الميداني.

تُبرز المراجعة الدوائية خصائص السليمارين المضادة للأكسدة والمضادة للالتهاب والمحسنة لحساسية الإنسولين، والتي تساهم في تحسين التحكم في نسبة السكر في الدم. أظهر استبيان كمي شمل 100 مريض مصاب بالسكري و10 مقدمي رعاية صحية أن 76٪ من المرضى كانوا على علم بالحرشف البري، إلا أن 3٪ فقط قد استخدموه لإدارة مرض السكري. من بين المستخدمين، أشار الجميع إلى انخفاض سكر الدم الصائم دون آثار جانبية، رغم عدم استشارة الطبيب. بالإضافة إلى ذلك، أعرب 79٪ من المرضى عن استعدادهم لاستخدام الحرشف البري إذا تم اعتماده طبيًا. أما بين الأطباء، فقد كان 50٪ على دراية بفوائده المحتملة، لكن لم يوصِ به أي منهم بسبب عدم كفاية الأدلة السريرية. ومع ذلك، رأى 70٪ أنه آمن بشروط وأبدوا استعدادًا لاستخدامه مستقبلًا في حال توفر إثباتات علمية أقوى.

وفي الختام، على الرغم من أن السليمارين يُظهر آليات واعدة كمضاد للسكري، فإن دمجه في الممارسة السريرية يتطلب تجارب سريرية قوية، وإشرافًا تنظيميًا، وتوعية الأطباء لضمان تطبيقه بشكل قائم على الأدلة في رعاية مرضى السكري.

الكلمات المفتاحية: داء السكري، الحرشف البري (Silybum marianum)، سيليمارين، مقاومة الإنسولين، الإجهاد التأكسدي، التحكم في سكر الدم.

Résumé

Le diabète sucré est un trouble métabolique chronique caractérisé par une hyperglycémie persistante due à une altération de la sécrétion d'insuline, à une résistance à l'insuline, ou aux deux. Étant donné la prévalence mondiale croissante du diabète, l'intérêt pour les thérapies complémentaires en parallèle des traitements conventionnels s'est accru. Ce mémoire étudie le potentiel thérapeutique de *Silybum marianum* (chardon-Marie), en se concentrant sur son constituant bioactif, la silymarine, à travers une approche intégrative combinant revue de la littérature, évaluation mécanistique et enquête de terrain.

La revue pharmacologique met en évidence les propriétés antioxydantes, anti-inflammatoires et sensibilisantes à l'insuline de la silymarine, contribuant ainsi à un meilleur contrôle glycémique. Une enquête quantitative auprès de 100 patients diabétiques et de 10 professionnels de santé a révélé que, bien que 76 % des patients connaissaient le chardon-Marie, seulement 3 % l'avaient utilisé pour la gestion du diabète. Parmi les utilisateurs, tous ont signalé une réduction de la glycémie à jeun sans effets indésirables, même si aucun n'avait consulté de médecin. De plus, 79 % des patients ont indiqué être prêts à utiliser le chardon-Marie s'il était approuvé médicalement. Parmi les médecins, 50 % connaissaient ses bénéfices potentiels, mais aucun ne l'avait recommandé en raison de preuves cliniques jugées insuffisantes. Néanmoins, 70 % le considéraient comme conditionnellement sûr et se montraient ouverts à son utilisation future sous réserve de validations scientifiques plus solides.

En conclusion, bien que la silymarine présente des mécanismes antidiabétiques prometteurs, son intégration en pratique clinique nécessite des essais cliniques rigoureux, un encadrement réglementaire et la formation des médecins afin d'assurer une application fondée sur des preuves dans la prise en charge du diabète.

Mots-clés : Diabète sucré, Chardon-Marie (Silybum marianum), Silymarine, Résistance à l'insuline, Stress oxydatif, Contrôle glycémique.

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PART I: LITERATURE REVIEW

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, insulin resistance, or both [1]. It is a major global health concern, with a continuously increasing prevalence that poses a significant burden on healthcare systems worldwide. According to the International Diabetes Federation (IDF), approximately 10.5% of adults aged 20-79—equating to 536.6 million individuals—were living with diabetes in 2021, with projections indicating a rise to 12.2% (783.2 million) by 2045 [2]. Additionally, a recent study published in *The Lancet* highlights that the number of adults diagnosed with diabetes has more than doubled over the past 30 years, now exceeding 800 million globally [3].

In 2022, an estimated 828 million adults worldwide had diabetes, marking a significant rise from 1990 [4]. The prevalence of diabetes increased in most countries, particularly in Asia, Africa, and Latin America, with the sharpest growth observed in low- and middle-income nations such as Malaysia, Pakistan, and Egypt. Conversely, prevalence remained stable in parts of Europe, Africa, and East Asia, while slight declines were noted in Japan, Spain, and France [2]. Despite growing awareness, treatment coverage remains inadequate. In 2022, 445 million adults (59% of those aged 30 and older) with diabetes were untreated, a figure 3.5 times higher than in 1990 [4]. Improvements in treatment access were noted in Europe, Latin America, Canada, South Korea, Russia, and parts of the Middle East. However, access remained critically low in Sub-Saharan Africa, South Asia, the Caribbean, and Pacific island nations [5]

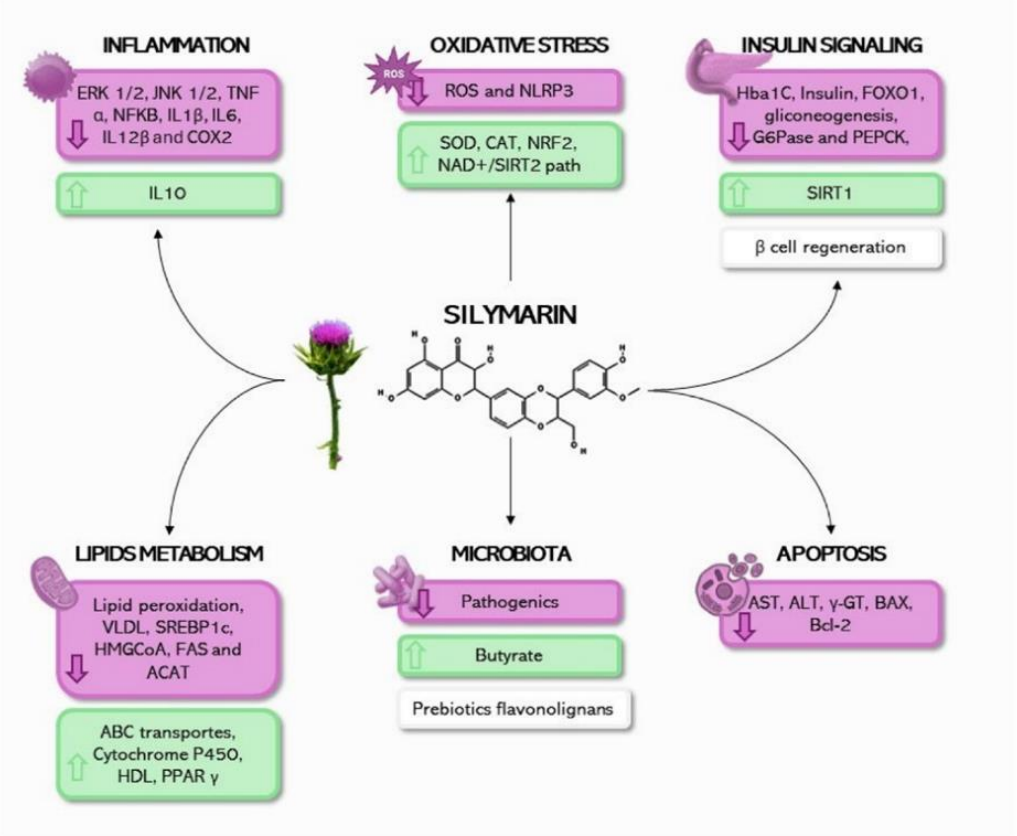

Figure 1: "Multifaceted Mechanisms of Silymarin in Metabolic Regulation and Diabetes Management [6]

Despite advances in medical treatments, the limitations of conventional therapies have led to a growing interest in alternative and complementary approaches for diabetes management. Among these, medicinal plants have emerged as promising candidates due to their bioactive compounds, which have been shown to exhibit significant anti-diabetic effects [7]. Flavonoids, alkaloids, terpenoids, and other plant-derived compounds have been extensively studied for their potential to regulate glucose metabolism, enhance pancreatic function, and improve insulin sensitivity [8] [9]. This shift towards natural remedies reflects a broader movement in medical research aimed at harnessing the therapeutic potential of botanical sources [10].

Milk thistle (*Silybum marianum*) is one such medicinal plant that has gained attention for its potential role in diabetes management. Traditionally known for its hepatoprotective properties, milk thistle has been used for centuries in the treatment of liver disorders [11]. Its primary bioactive component, silymarin, is a complex of flavonolignans with potent antioxidant, anti-inflammatory, and hepatoprotective properties. Recent studies suggest that silymarin may also exert beneficial effects on glucose metabolism, insulin sensitivity, and pancreatic function, making it a promising candidate for diabetes treatment [12]. Silymarin has been shown to reduce oxidative stress, a key contributor to diabetes pathogenesis, by scavenging free radicals and enhancing the activity of endogenous

antioxidant enzymes [13] [14]. It also modulates pro-inflammatory cytokines, improves lipid profiles, enhances insulin secretion, and improves glucose uptake in peripheral tissues [15] [16]. Animal and clinical studies have demonstrated that silymarin supplementation can lower fasting blood glucose levels, reduce glycated hemoglobin (HbA1c), and improve metabolic health in diabetic patients [16].

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Milk thistle (Silybum marianum) is one such medicinal plant that has gained attention for its potential role in diabetes management. Traditionally known for its hepatoprotective properties, milk thistle has been used for centuries in the treatment of liver disorders [18] [19]. Its primary bioactive component, silymarin, is a complex of flavonolignans with potent antioxidant, anti-inflammatory, and hepatoprotective properties. Recent studies suggest that silymarin may also exert beneficial effects on glucose metabolism, insulin sensitivity, and pancreatic function, making it a promising candidate for diabetes treatment [20] .

The pharmacological actions of milk thistle in diabetes are attributed to multiple mechanisms. Silymarin has been shown to reduce oxidative stress, a key contributor to diabetes pathogenesis, by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes [21] [22].

Additionally, it exerts anti-inflammatory effects by modulating pro-inflammatory cytokines, which play a crucial role in insulin resistance and pancreatic β-cell dysfunction [23]. Some studies have also indicated that milk thistle can influence lipid metabolism, contributing to improved lipid profiles in diabetic individuals, which is essential for reducing the risk of cardiovascular complications associated with the disease [24].

Moreover, research has suggested that milk thistle may enhance insulin secretion and improve glucose uptake in peripheral tissues, thereby promoting better glycemic control. Animal and clinical studies have demonstrated that silymarin supplementation can lower fasting blood glucose levels, reduce glycated hemoglobin (HbA1c), and improve overall metabolic health in diabetic patients [25]. These findings highlight the potential of milk thistle as a complementary therapy for diabetes management, particularly for individuals seeking natural alternatives to conventional pharmacotherapy.

However, while the therapeutic potential of milk thistle in diabetes management is promising, further research is necessary to elucidate its mechanisms of action, optimal dosages, and long-term efficacy. Clinical trials evaluating its safety and effectiveness in diverse populations will be crucial for establishing evidence-based recommendations for its use in diabetes treatment. Additionally, understanding possible interactions with

conventional anti-diabetic medications is essential to ensure its safe integration into clinical practice [26].

In summary, diabetes remains a significant global health challenge, necessitating innovative and holistic approaches to its management. Medicinal plants such as milk thistle offer a promising avenue for developing novel therapeutic strategies. As scientific interest in natural compounds continues to grow, understanding the potential role of milk thistle in diabetes treatment may pave the way for more effective and integrative approaches to managing this chronic condition.

CHAPTER 01 :MILK THISLE

1.Milk Thistle (Silybum marianum) and Silymarin in Diabetes Management

Among the various medicinal plants under investigation, Silybum marianum, commonly known as milk thistle, has received considerable scientific attention. Traditionally recognized for its hepatoprotective properties, milk thistle owes its therapeutic effects primarily to silymarin, a complex of flavonolignans with potent antioxidant and anti-inflammatory activities [27], [28]. Recent research indicates that silymarin may offer specific benefits for individuals with diabetes by improving glycemic control, enhancing insulin sensitivity, preserving pancreatic β-cell function, and reducing oxidative stress and inflammation—key pathological processes in the development of type 2 diabetes [29] [30] [31]

Figure 2: Silybum marianum, commonly known as milk thistle [27]

1.2. Pharmacological Actions of Silymarin in Diabetes

The pharmacological actions of milk thistle in diabetes are attributed to multiple mechanisms. Silymarin has been shown to reduce oxidative stress, a key contributor to diabetes pathogenesis, by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes [32] [33]. Additionally, it exerts anti-inflammatory effects by modulating pro-inflammatory cytokines, which play a crucial role in insulin resistance and pancreatic β-cell dysfunction [34]

Figure 3: Milk Thistle Dietary Supplements [32]

1.3. Effects on Lipid Metabolism and Glycemic Control

Some studies have indicated that milk thistle can influence lipid metabolism, contributing to improved lipid profiles in diabetic individuals, which is essential for reducing the risk of cardiovascular complications associated with the disease [35]. Moreover, research has suggested that milk thistle may enhance insulin secretion and improve glucose uptake in peripheral tissues, thereby promoting better glycemic control. Animal and clinical studies have demonstrated that silymarin supplementation can lower fasting blood glucose levels, reduce glycated hemoglobin (HbA1c), and improve overall metabolic health in diabetic patients [36] [37]

Figure 4: milk thistle's seeds [37]

1.4. Clinical Evidence for Milk Thistle in Diabetes

Both animal models and clinical trials have demonstrated the hypoglycemic and lipid-lowering effects of silymarin. Studies have reported significant reductions in fasting blood glucose, HbA1c, and serum lipid concentrations following silymarin administration, suggesting its role as a potentially effective adjunct in diabetes management [38] [39]

This thesis aims to provide a comprehensive exploration of the therapeutic potential of milk thistle (Silybum marianum), with a particular focus on its active constituent silymarin, in the context of type 2 diabetes mellitus. By synthesizing evidence from both preclinical and clinical studies, the research aims to elucidate the biochemical and physiological mechanisms by which silymarin may influence key metabolic processes, including glucose homeostasis, insulin sensitivity, and pancreatic β-cell preservation [40] [41] [42]

In light of the ongoing global burden of diabetes and the limitations associated with conventional pharmacotherapies—such as adverse effects, long-term inefficacy, and economic barriers—there is a pressing need for complementary strategies that offer both efficacy and safety [43]. Medicinal plants, and particularly milk thistle, have emerged as promising candidates due to their rich composition of bioactive flavonolignans with established antioxidant and anti-inflammatory properties [40] [41] [42] [43] [44]

This study contributes to a growing field of integrative metabolic research by critically evaluating silymarin's impact on glycemic control and its potential to mitigate complications related to type 2 diabetes. By addressing current gaps in the literature and emphasizing milk thistle's favorable safety profile and historical use in traditional medicine, this thesis supports the integration of evidence-based botanical interventions into modern therapeutic frameworks. In doing so, it aims to advance the understanding of plant-based adjuncts as viable components in the holistic and personalized management of diabetes [40], [45]

2. Historical studies

Milk thistle has a long-standing history of medicinal use. Theophrastus (c. 371–287 BCE), a Greek philosopher and student of Aristotle, referred to milk thistle under the name Pternix. Later, Pliny the Elder (23–79 CE) and Dioscorides (40–90 CE) documented its therapeutic applications in classical medical texts. By the 16th century, milk thistle gained popularity in Europe for its purported efficacy in treating liver and gallbladder disorders. In 1652, the noted English herbalist Nicholas Culpeper highlighted its effectiveness for ailments of the liver and spleen in his work The English Physician [45].

The plant was brought to the Americas by early European settlers, where it continued to be used in traditional medicine. In the early 19th century, the Eclectics, a group of herbal practitioners, employed milk thistle extracts to manage disorders of the liver, spleen, kidneys, and even to regulate menstruation. However, its popularity waned until the mid-20th century. A resurgence of interest in milk thistle occurred in the 1960s, particularly in

Germany, where researchers began to investigate its hepatoprotective effects and the pharmacological potential of its primary constituent, silymarin [46]

3. Ecological studies

Milk thistle is a resilient plant capable of thriving across a variety of climates, including northern temperate zones and arid, subtropical environments. Its adaptability has made it suitable for cultivation in diverse geographic conditions. Studies have shown that environmental factors such as temperature and soil type influence the concentration of active compounds in the plant. Specifically, silybin, the primary active flavonolignan in silymarin, tends to accumulate in higher quantities under subtropical conditions compared to temperate ones, suggesting a temperature-dependent biosynthetic pathway [47]

The plant is well-suited to various soil types and often appears in disturbed areas such as roadsides, garbage dumps, and abandoned agricultural fields [48]. Despite its medicinal value, milk thistle is frequently classified as a noxious weed due to its invasive behavior and competition with crops for essential resources like water and nutrients [49], [50]

Moreover, milk thistle's ability to absorb and accumulate nitrate poses a toxic risk to livestock, particularly when consumed in its young and wilted form [51]. The concentration of silymarin in the plant's fruits also varies depending on genotype and growing conditions, with optimal yields obtained in nutrient-rich, nitrogen-dense environments such as dairy yards or poultry waste zones [47], [49].

4. Botanical studies:

Table 1: Scientific classification of Silybum marianum [52]

Kingdom:	**Plantae**
***Clade*:**	**Tracheophytes**
***Clade*:**	**Angiosperms**
***Clade*:**	**Eudicots**
***Clade*:**	**Asterids**
Order:	**Asterales**
Family:	**Asteraceae**
Subfamily:	**Carduoideae**
Tribe:	**Cardueae**
Subtribe:	**Carduinae**
Genus:	***Silybum***

-Milk thistle, scientifically known as Silybum marianum (L.) Gaertn., is an herbaceous plant that has been used for centuries in traditional medicine [53].

- Scientific Name: Silybum marianum (L.) Gaertn.
- Other Common Names: Our Lady's Milk Thistle, Blessed Thistle [53].

The name "milk thistle" is attributed to the milky sap that exudes from the leaves when they are crushed [54]

-Milk thistle is characterized by its distinctive morphological features:

- Growth Habit: Herbaceous [53]
- Life Cycle: Annual [53]

This plant is known for its erect growth, large prickly leaves with white veins, and purple to reddish-purple flowers [55]

Native Distribution: Milk thistle is native to the Mediterranean region, ranging from southern Europe to Afghanistan [53]

4.1 .Scotch Thistle (Onopordum acanthium)

Although Milk Thistle and Scotch Thistle belong to the same family and appear similar at first glance, they differ significantly in leaf color and texture, flower morphology, and especially in seed composition. Milk thistle leaves show unique white veining, and its seeds are pharmacologically important due to silymarin. In contrast, Onopordum acanthium is more woolly, spiny, and lacks the medicinal compounds found in Milk Thistle.

4.1.1. Leaves

Table 2: Comparative Morphology of Leaves Between Silybum marianum and Onopordum acanthium

Feature	**Milk Thistle (*Silybum marianum*)**	**Scotch Thistle (*Onopordum acanthium*)**
Shape	Large, lobed or pinnatisect	Deeply lobed or pinnatifid
Color	Bright green with distinctive white marbling along the veins (milky appearance)	Dull green to grayish-green with a dense woolly covering (tomentose)
Texture	Smooth surface, less hairy	Very spiny and densely hairy or woolly
Margins	Wavy with sharp spines on the lobes	Strongly spiny margins and leaf tips
Arrangement	Rosette at base in young plant, alternate along stem	Rosette in first year, alternate along branching stems

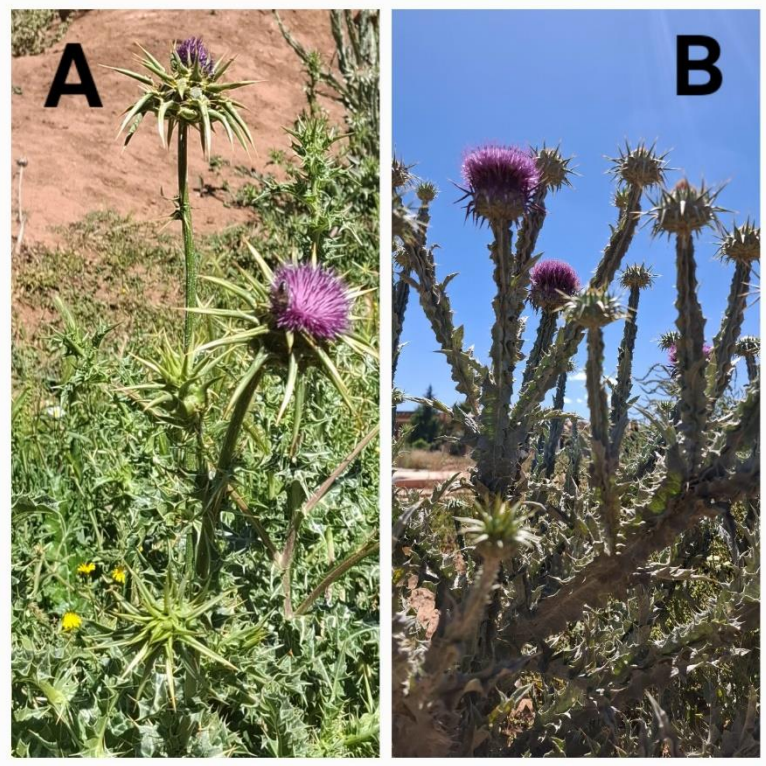

Figure 5: Morphological traits of Silybum marianum leaves (A) and Scotch Thistle (Onopordum acanthium) leaves (B).

4.1.2. Flowers

Table 3: Comparative Morphology of Leaves Between Silybum marianum and Onopordum acanthium

Feature	**Milk Thistle (*Silybum marianum*)**	**Scotch Thistle (*Onopordum acanthium*)**
Color	Bright purple to pinkish-purple	Purple to lilac
Flower Head	Solitary, large, surrounded by spiny bracts	Also solitary or in small clusters, surrounded by woolly and spiny bracts
Involucre Bracts	Hard, triangular, strongly spiny, often curved outward	Woolly, also spiny but broader and more papery in appearance
Flowering Season	Late spring to summer (May–July)	Mid to late summer (June–August)

Figure 6 : Morphological traits of Silybum marianum flowers (A) and Scotch Thistle (Onopordum acanthium) flowers (B).

4.1.3. Seeds (Achenes)

Table 4 : Differences in Seed Morphology and Composition Between Silybum marianum and Onopordum acanthium

Feature	**Milk Thistle (*Silybum marianum*)**	**Scotch Thistle (*Onopordum acanthium*)**
Shape	Oblong, smooth achenes	Oblong but more angular
Color	Grey to black with a shiny surface	Grayish to brown, duller surface
Pappus	Long, silky hairs for wind dispersal	Also has pappus but often shorter and coarser
Use	Rich in silymarin, used medicinally	Not known for any specific pharmacological compound

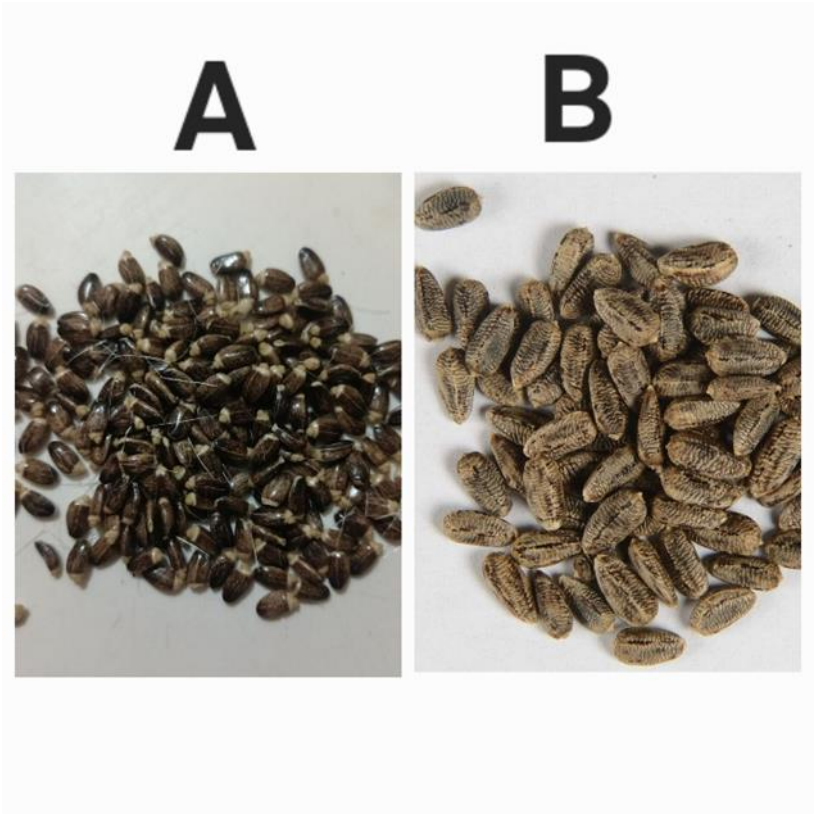

Figure 7: Differences in Seed Morphology Between Silybum marianum A and Onopordum acanthium B

5. Description

5.1. Morphology

There is great variability in the morphological characteristics of S. marianum, and a comprehensive list of descriptors for the species was compiled in 2016 [56]. The plant has a glabrous or slightly downy stem that is erect and branched in the upper part [57]. Depending on different soil fertility and environmental conditions, individual stem heights can vary between 40 and 200 cm. The basal leaves are alternate, large, and glabrous with spiny margins (Figure 3A). Leaf size typically ranges from 50 to 60 cm in length and 20 to 30 cm in width [58]. In addition, white veins along the upper side of the leaf are a distinctive feature of the species, although the presence of individuals with uniformly green leaves has been reported [59]. The stem leaves are smaller than those of the rosette.

Each stem, including those of lateral branches, ends with a flower head about 5 cm in diameter [57], raised above the leaves. The flower is usually red-purple, but a white-flowered genotype was collected and studied by Szilágyi and Tétényi in the early 1970s; the occurrence of white-flowered genotypes has also been reported in Israel [60]. The inflorescence heads are surrounded by spiny bracts (Figure 3B). The numerous florets are hermaphroditic, with a tubular, five-lobed corolla.

The fruits ("seeds") are achenes, characterized by a long white pappus; their color ranges from black to glossy brown, but grey ones with spots are also common. The weight of 1000 seeds varies between 20 and 30 g [61]. Depending on the genotype and growth conditions, each flower head can produce from about 65-100 to about 190 seeds, making more than 6000 seeds per plant, of which 94% are viable [61].

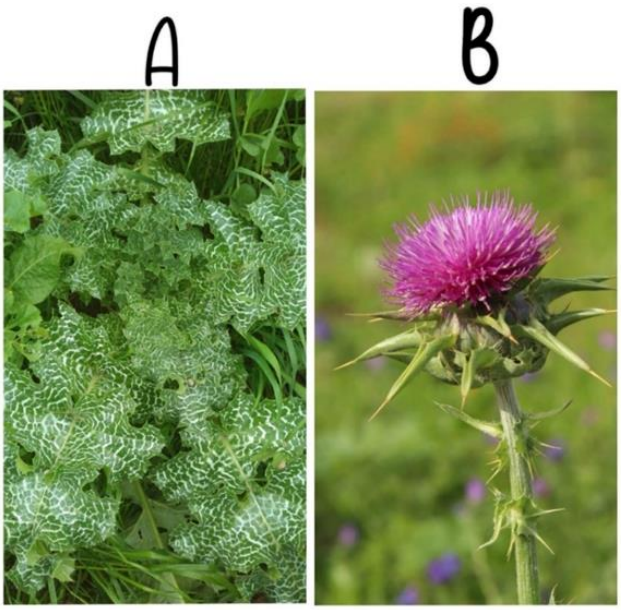

Figure 8 : Morphological traits of Silybum marianum leaves (A) and a red-purple flower head with spiny bracts (B). (Photos: G. Di Miceli).

5.2. Biology and Physiology

Milk thistle is generally classified as an annual, although it may be a biennial [62]. In the wild, seeds germinate in the fall and flower the following summer, resulting in a vegetative cycle of 8-9 months. The phenological stages of milk thistle can be described using a two- or three-digit BBCH (Biological Bundesanstalt, Bundessortenamt und Chemische Industrie) coding system, scaled from 0 (seed germination) to 9 (plant senescence) [63]. Accordingly, plant growth can be divided into four stages: germination (BBCH stage 0), growth period (BBCH stages 1-4), flowering (BBCH stages 5-6), and seed development to maturity (BBCH stages 7-8) [62].

Emergence begins 1 to 3 weeks after sowing, depending mainly on temperature [64]. During the vegetative stage, S. marianum develops a large rosette and overwinters in this form. At this stage, it is easily identified by its characteristic spiny and variegated leaves and can compete strongly with neighboring annuals [65]. The flowering stage includes the development of inflorescences from the central stem and axillary branches, with flowering occurring from April to May [66]. Plant height and branching are influenced by weather conditions, soil fertility, and sowing density.

The final stage involves seed growth and ripening, usually maturing in July. When the fruits on the lateral branches are ripe, the plant appears completely dead and dry [66].

6.Taxonomy and Chemical Structure

At physiological maturity, the seed of S. marianum contains about 7% water, 20-30% lipids, 20-30% proteins, 0.038% tocopherols, 0.63% sterol, and other compounds such as 3-deoxyflavonolignan mucilage [67].

According to Malekzadeh et al. [68], the most abundant oil components are oleic (36.7%) and linoleic (39.7%) acids. Other less abundant fatty acids include palmitic (10.2%), stearic (6.9%), arachidic (3.6%) and behenic (2.5%) acids. Environmental factors, especially drought stress, can shift the fatty acid profile toward increased linoleic acid [68]. Soil fertilization also positively affects the content of unsaturated fatty acids [69].

The oil contains significant levels of phospholipids, phytosterols, and vitamin E, enhancing its nutritional value [70].

6.1. Silymarin

Silymarin, responsible for the therapeutic properties of milk thistle, was first isolated by Wagner et al. It is a mixture of several flavonolignans including silycristin, silydianin, silybin (A and B), and isosilybin (A and B) [71]. Silybin makes up 50-70% of silymarin and is the most bioactive component [72]

Silybin (synonymous with silibinin), the most bioactive component of milk thistle extract , was first isolated and identified by Pelter and Hänsel . It is considered safe, with no serious side effects except for mild gastrointestinal disturbance, and due to its very low

overall toxicity, the US Food and Drug Administration (FDA) has approved its use as a phytomedicine for the treatment of liver disease [72].

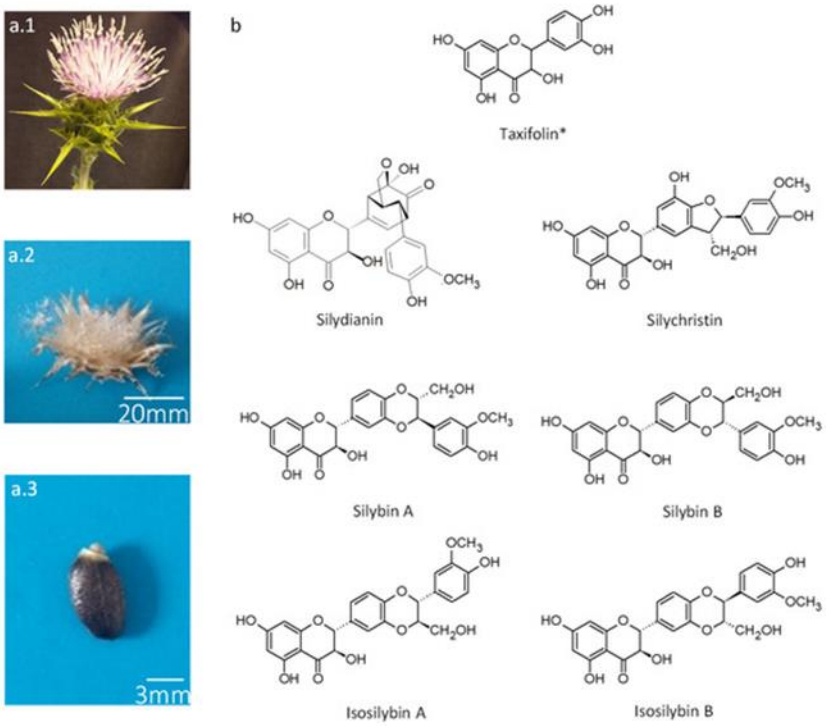

Figure 9 : Major constituents of silymarin. (Adapted from [73]).

High intraspecific variability in the relative amounts of silymarin constituents has been reported by many authors . Martinelli et al showed that the amount of each flavonolignan in the silymarin mixture was the most variable trait among 26 accessions collected from different locations. Research on the phytochemical variability of milk thistle first allowed the identification of two high-silybin and high-silydianin chemotypes . In a later work , the same research group listed three chemotypes (termed A, B, and C) within Italian wild populations, with chemotype C being the result of hybridization between A and B [74].

6.2. Biosynthesis and Accumulation

Considerable research has been devoted to the study of the biosynthetic process of milk thistle flavonolignans. In the first step, they are synthesized via the phenylpropanoid pathway by the oxidative radicalization of two precursors, namely a flavonoid (mostly taxifolin) and coniferyl alcohol; the final step consists of the coupling of these two radicals . The biosynthesis of taxifolin takes place in the flower, then it is transferred to the pericarp where the synthesis of the silymarin components takes place [75]. The main storage site for silymarin is the seed integument, while only trace amounts are present in the pericarp layer . However, the amount and accumulation of silymarin in the seed integument of milk thistle is highly variable and strongly influenced by environmental conditions; research has shown

that drought stress conditions can enhance the biosynthesis of this active substance in plant tissues , and that its accumulation in fruits is related to the growth phase and lignification of the seed coat [76]. According to the European Pharmacopoeia and the United States National Formulary, mature fruits of S. marianum must contain at least 1.5-2% silymarin on dry matter . However, there is evidence that seed silymarin content can be highly variable depending on genotype and growth conditions, as values ranging from 0.62 to 2.25% , 2 to 4% , and sometimes as high as 4.2% and 6% [76] have been reporte

Due to the high pharmaceutical relevance of silymarin and the increasing demand of pharmaceutical companies for a steady and homogeneous supply of silymarin compounds, recent efforts have focused on different approaches to maximize and stabilize its production, including effective extraction methods and biotechnological production [74]

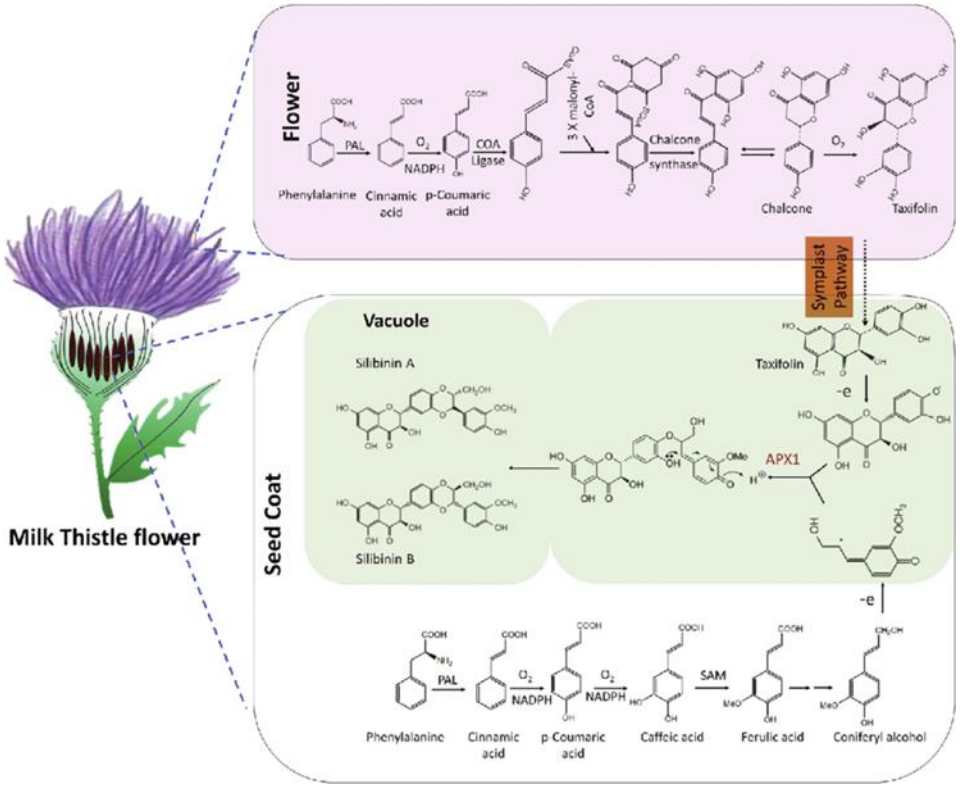

Figure 10: Sequential steps in the biosynthesis of silymarin components including silibinin from S. marianum [77].

6.3. Extraction Methods

Due to the high lipid content, silymarin extraction typically involves a two-step process: defatting with n-hexane, followed by methanol extraction [78]. Pressurized Liquid Extraction (PLE) and modern techniques such as microwave-, ultrasound-, and enzyme-assisted extraction improve efficiency and reduce toxicity.

However, this extraction procedure has several drawbacks, mostly related to the excessive duration of the process and the high toxicity of hexane. Therefore, alternative methods for silymarin extraction have been investigated. A significant shortening of the

process can be achieved by using the technique of Pressurized Liquid Extraction (PLE) [78], which allows avoiding the preliminary defatting otherwise required in the traditional method. In addition, new technologies such as microwave-assisted, ultrasound-assisted, and enzyme-assisted extraction have been investigated to increase the extraction yield of silymarin [79].

6.4. Biotechnological Production

Biotechnological methods offer a stable alternative for silymarin production. While early in vitro cultures showed limited yield, mass cultivation using hairy root bioreactors offers promise . Further optimization is required to scale these methods for industrial use

This approach could be an effective alternative method to make silymarin production continuous and stable over time, while overcoming the problems associated with conventional open-field production [79] as well as the high intraspecific variability of silymarin seed content . Tissue and cell culture, as well as in vitro regeneration techniques, with or without the addition of elicitors to the growth medium , have been used to this end. Studies using in vitro cultures of S. marianum began in the late 1970s and continued, with contrasting results, throughout the last decades of the twentieth century to the present . In most cases, in vitro techniques allowed low yields of silymarin to be compared with field production [80]. Better results have been obtained using a bioreactor system consisting of mass cultivation of S. marianum hairy roots for large-scale silymarin production. However, this technique is still far from industrial application, and specialized cultivation of the starting plant material is still the most reliable way to produce silymarin. For future development, further efforts are needed to optimize the physical and chemical conditions for in vitro cultures of milk thistle in bioreactor systems [71].

CHAPTER 2: ANTIDIABETIC POTENTIAL OF MILK THISTLE

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of diabetes has risen significantly over recent decades, wih the International Diabetes Federation estimating that approximately 537 million adults were living with diabetes in 2021—a number projected to rise to 643 million by 2030 [81]. Type 2 diabetes mellitus (T2DM), the most common form, is often associated with obesity, sedentary lifestyles, and dietary habits.

Conventional treatments for diabetes primarily focus on glycemic control through lifestyle modification, oral hypoglycemic agents, and insulin therapy. However, these treatments may have side effects and are often insufficient in preventing long-term complications such as nephropathy, neuropathy, and cardiovascular diseases. As a result, there is growing interest in complementary and alternative medicines (CAMs) to support diabetes management [82].

Among the various herbal remedies, milk thistle (Silybum marianum) has gained attention due to its potential therapeutic properties. Traditionally used for liver disorders, milk thistle contains silymarin, a complex of flavonolignans with antioxidant, anti-inflammatory, and insulin-sensitizing effects [83]. Emerging studies suggest that milk thistle may improve glycemic control and reduce insulin resistance, making it a promising adjunctive therapy in diabetes management.

The purpose of this thesis is to explore the potential role of milk thistle in the management of diabetes, with a focus on its pharmacological mechanisms, efficacy, and safety profile based on preclinical and clinical evidence. Understanding the therapeutic potential of milk thistle may offer insights into its role as a complementary strategy for improving metabolic health in diabetic patients.

1 . Phytochemical Composition of Milk Thistle Key Bioactive Compounds

Silymarin is a flavonoid-rich extract derived from the seeds and fruits of the milk thistle plant (Silybum marianum (L.) Gaertn.). It is available as a dietary supplement and, in some

Silymarin is a flavonoid-rich extract obtained primarily from the seeds and fruits of the milk thistle plant (Silybum marianum (L.) Gaertn.) and is widely recognized for its medicinal properties [84], [85]. Its major active component is silybin (also known as silibinin), a flavonolignan known for its antioxidant, anti-inflammatory, and cytoprotective activities [86], [87]. Silymarin also contains other bioactive molecules, including taxifolin-derived flavonolignans, flavonoids, and polyphenols [88], [89].

Traditionally used for liver ailments, silymarin is approved in some countries as a therapeutic agent and is also available as an over-the-counter dietary supplement [84]– [87]. Apart from hepatoprotective effects [89]– [90], silymarin has demonstrated potential benefits in various inflammatory and neurodegenerative diseases, such as rhinitis, arthritis, Alzheimer’s disease, and Parkinson’s disease [91] [92] [93] [94]. Its antioxidant activity has also been well documented [95].

The bioactive flavonolignans of silymarin, including silybinin A & B, isosilybinin A & B, silychristin, and silydianin, vary in concentration depending on the plant part (most concentrated in the seeds), geographical origin, and environmental factors [96]– [97]The plant's therapeutic value is attributed to its rich content of antioxidants and polyphenolic compounds.

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder marked by insulin resistance and β-cell dysfunction, resulting in persistent hyperglycemia [98] [99] [100], [101] [102]. While conventional treatments are available, there is growing interest in plant-derived polyphenols, especially flavonoids, for managing diabetes due to their antioxidative and anti-inflammatory effects [102]. Studies have shown that silymarin and its constituents can reduce oxidative stress, enhance insulin sensitivity, and protect β-cells from glucotoxicity and lipotoxicity [103] [104].

Mechanistically, silymarin exerts its antidiabetic effects by upregulating endogenous antioxidant enzymes like superoxide dismutase and catalase, and by inhibiting lipid peroxidation [95], [105]. Clinical data suggest silymarin is generally well-tolerated, with minimal adverse effects in both healthy individuals and diabetic patients [92].

The main bioactive component of the medicinal plant milk thistle (Silybum marianum) is silymarin, a complex mixture of flavonolignans including silybinin A and B (SBN A&B), isosilybinin A and B (ISBN A&B), silychristin (SCN), and silydianin (SDN) [106], [107]. Silymarin has long been recognized as a traditional Chinese herbal remedy [108]. It is present in the seeds, fruit, and leaves of the milk thistle plant, with the highest concentrations found in the seeds [109]. The silymarin content in milk thistle fruit varies depending on the plant variety, geographical location, and environmental conditions in which it is cultivated [110]. Due to the presence of phytoconstituents such as antioxidants and phenolic compounds, milk thistle has been used in the management of various diseases [106].

2. Distinct Mechanisms Underlying the Antidiabetic Action of Milk Thistle

2.1. Hepatic Effects

The liver plays a central role in metabolism and detoxification and is highly vulnerable to injury due to its constant exposure to xenobiotics entering via the gastrointestinal tract. These compounds are first transported to the liver, which heightens the risk of hepatic disorders ranging from acute hepatitis to hepatocellular carcinoma. Pathogenic processes such as apoptosis, necrosis, inflammation, immune responses, fibrosis, ischemia, altered gene expression, and aberrant regeneration contribute to liver damage [111].

Silymarin has long been utilized as a hepatoprotective agent, although its precise mechanisms remain incompletely understood. Its pharmacological effects are attributed to antioxidant, immunomodulatory, antifibrotic, antiproliferative, and antiviral activities. It is rapidly metabolized by the liver and primarily excreted in bile. In order to effectively manage hepatic inflammation, high or repeated oral dosing is typically required [112].

2.1.1. Inhibition of Gluconeogenesis and Glycogenolysis

Silymarin and its principal active compound, silybinin, exert regulatory effects on hepatic glucose homeostasis by targeting gluconeogenesis and glycogenolysis, two pivotal processes in the pathophysiology of metabolic disorders such as type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) [113].

2.1.1.1 Gluconeogenesis Suppression

Silymarin downregulates the expression of key gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), via mechanisms involving AMPK activation and enhancement of the IRS-1/PI3K/Akt signaling pathway, leading to a reduction in hepatic glucose production [114]. In diabetic rat models, administration of silymarin (200 mg/kg/day) reduced fasting blood glucose levels by approximately 30%, an effect associated with suppressed PEPCK expression [115].

2.1.1.2. Glycogenolysis Inhibition

Silybinin has been shown to directly inhibit glycogen phosphorylase, thereby preserving hepatic glycogen content [116]. In vitro studies on hepatocytes revealed a 40–50% reduction in glycogenolytic activity following treatment with silybinin at 100 μM concentrations [117].

2.1.1.3. Synergistic Effects

The combined inhibition of gluconeogenesis and glycogenolysis improves hepatic insulin sensitivity, as evidenced by decreased HOMA-IR indices in clinical settings [118].

2.1.1.4.Clinical Relevance

A 2020 meta-analysis found that silymarin supplementation at 600 mg/day led to a significant reduction in HbA1c levels (−0.8%) among patients with T2DM [119]. However, most of the current evidence is derived from preclinical studies, and human data remain relatively limited [120].

2.1.2. Modulation of Lipid Metabolism and Steatosis

Elevated plasma levels of free fatty acids are positively correlated with NAFLD severity. Agents that mitigate hepatocellular injury caused by these lipids hold therapeutic potential [121].

In rat models of carbon tetrachloride-induced liver injury, ethanolic silymarin seed extract (100 mg/kg bw) significantly reduced liver enzyme levels. Furthermore, the ethyl acetate fraction showed improved glutathione content and HDL/LDL profiles [122].

Palmitate-induced inhibition of Akt kinase triggers hepatocyte death in HepG2 cells. Silymarin counters this inhibition, demonstrating a hepatoprotective effect distinct from its antioxidant function [123].

2.1.3. Redox Balance and Inflammatory Signaling

Silymarin exerts antioxidative effects by elevating intracellular glutathione, thereby preventing lipid peroxidation and stabilizing cell membranes under xenobiotic stress. It also exhibits corticosteroid-like activity by modulating nuclear protein expression and inhibits

collagen deposition by preventing the transformation of hepatic stellate cells into myofibroblasts. Additionally, silymarin promotes ribosomal protein synthesis through RNA polymerase I stimulation [124].

Silybinin has been observed to inhibit hepatic expression of pro-inflammatory cytokines such as IL-2, IL-4, IFN-γ, and TNF-α, while also lowering aminotransferase levels and reducing hepatocyte apoptosis [112].

In a murine model of alcoholic liver disease, characterized by oxidative stress and inflammation, silymarin induced TNF production, reduced serum ALT activity, curtailed lipid peroxidation, and enhanced intracellular GSH levels [124]

Pretreatment with silymarin in male mice modulated oxidative stress, cytoskeletal remodeling, inflammation, apoptosis, and metabolic gene expression triggered by pyrogallol exposure. Transcriptomic analysis identified 79 significantly altered genes (27 upregulated, 52 downregulated) [125]

Additionally, silymarin has been shown to reduce hepatic amiodarone concentrations and ameliorate lysosomal phospholipidosis [124].

2.1.4. Clinical Data in NAFLD and T2DM

Silymarin and silybinin protect hepatic tissue from damage induced by various toxins, including ethanol, carbon tetrachloride, cisplatin, thioacetamide, thallium, D-galactosamine, and acetaminophen in preclinical models [111].

In a study using a diethylnitrosamine-induced liver injury model in rats, silymarin (50 mg/kg, orally for 30 days) significantly reversed histopathological alterations, offering notable hepatoprotection [126].

Fibrosis is a common sequela of chronic liver infection.reported that silymarin suppressed replication of the HCV genotype 2a (JFH1) in hepatoma cells by inhibiting HCV RNA-dependent RNA polymerase [127]. Ongoing Phase II trials sponsored by the NCCAM are evaluating its efficacy in chronic hepatitis C.

In a two-year clinical study on alcoholic liver cirrhosis, patients receiving silymarin (150 mg thrice daily) showed no significant improvement in survival or disease progression compared to controls [128].

Currently, silymarin is primarily employed for Amanita phalloides (death cap) poisoning. Its protective effects are mediated by halting enterohepatic recirculation of α-amanitin, and inhibiting its cellular uptake and membrane transport [126]

A retrospective review of 205 Amanita poisoning cases found zero fatalities in 16 patients treated with intravenous silybinin (20–50 mg/kg/day), although clinical outcomes across studies remain inconsistent [126]

Further research indicates that silymarin conjugates are excreted via MRP2, a hepatic transporter whose expression is altered in chronic liver disease. Silydianin, a glucuronidated silymarin component, may serve as an MRP2 substrate probe [129].

Animal and human studies consistently show that silymarin's most potent hepatoprotective effects correlate with higher dosing concentrations [112].

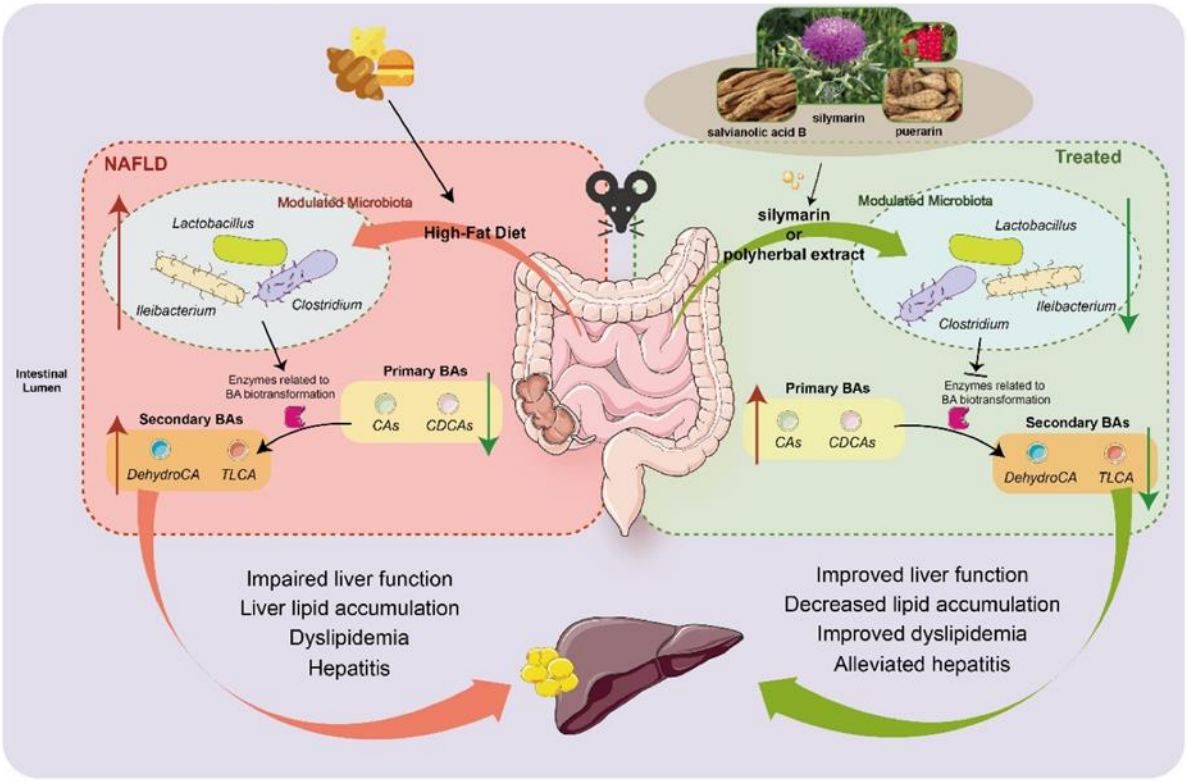

Figure 11: Metabolism of Silymarin to Improve High-Fat Diet-Induced NAFLD in Mice [112]

2.2. Skeletal Muscle Effects

2.2.1. Enhancement of Insulin Signal Transduction

Silymarin, particularly its active component silibinin, has been studied for its potential effects on insulin signaling in skeletal muscle. In insulin-resistant C2C12 myotubes induced by palmitate, silibinin treatment prevented the decrease in insulin-stimulated glucose uptake and the downregulation of GLUT4 translocation. This suggests that silibinin can enhance insulin signal transduction by maintaining the functionality of key components in the insulin signaling pathway, such as the insulin receptor substrate-1 (IRS-1) and Akt phosphorylation [130].

2.2.2. PI3K/Akt Pathway Restoration and GLUT4 Translocation

The PI3K/Akt pathway plays a pivotal role in mediating insulin's effects on glucose uptake in skeletal muscle. Silibinin has been shown to restore this pathway's functionality in insulin-resistant conditions. Specifically, silibinin treatment in palmitate-induced insulin-resistant C2C12 myotubes suppressed the decrease in insulin-stimulated Akt Ser473

phosphorylation. This restoration of Akt activity is crucial for the translocation of GLUT4 to the plasma membrane, facilitating glucose uptake into the muscle cells [130].

2.2.3. Contextual Considerations and Model-Specific Effects

While silibinin exhibits beneficial effects in certain models of insulin resistance, it's important to note that silymarin's effects can vary depending on the context. For instance, in Wistar rats, oral administration of silymarin induced insulin resistance and increased the expression of phosphatase and tensin homolog (PTEN) in skeletal muscle and liver tissues. This upregulation of PTEN, a negative regulator of the PI3K/Akt pathway, led to decreased Akt phosphorylation and impaired insulin signaling. These findings highlight the complexity of silymarin's effects and underscore the importance of considering specific physiological and pathological contexts when evaluating its potential therapeutic applications [131].

2.3. Adipose Tissue Modulation

2.3.1. Anti-adipogenic Actions in Preadipocytes

Silymarin exhibits notable anti-adipogenic properties, particularly in preadipocyte models such as 3T3-L1 cells. Treatment with silymarin or its active flavonolignan, silibinin, significantly inhibits the differentiation of preadipocytes into mature adipocytes by downregulating key adipogenic transcription factors, including peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer-binding protein alpha (C/EBPα) [132], [133]. Furthermore, silymarin reduces intracellular lipid accumulation and triglyceride synthesis, likely through suppression of adipogenic gene expression [134]. Mechanistically, these effects are associated with the inhibition of the MAPK/ERK and PI3K/Akt pathways, which are critical for adipogenesis and insulin signaling [135].

2.3.2. Regulation of Adipokine Profiles and Insulin Sensitivity

Beyond direct anti-adipogenic effects, silymarin influences adipose tissue function by modulating adipokine secretion. In both in vitro and animal models, silymarin has been shown to reduce levels of pro-inflammatory adipokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), while enhancing adiponectin expression—a key insulin-sensitizing adipokine [136], [137]. This shift in adipokine profile is associated with improved insulin sensitivity and reduced systemic inflammation in models of obesity and T2DM [138]. The restoration of adiponectin and suppression of TNF-α contribute to enhanced glucose uptake and insulin receptor signaling in peripheral tissues [139].

2.3.3. In Vivo Effects on Adiposity and Inflammation

In vivo studies support the anti-obesity and anti-inflammatory effects of silymarin. Rodent models fed high-fat diets and treated with silymarin or silibinin exhibit significant reductions in body weight gain, visceral fat mass, and adipocyte hypertrophy [140], [141]. Histological analysis further reveals a decrease in macrophage infiltration and crown-like

structure formation in adipose tissue, indicating reduced adipose inflammation [142]. These improvements correlate with enhanced systemic insulin sensitivity and decreased circulating inflammatory cytokines [143]. Importantly, silymarin also mitigates hepatic steatosis, suggesting coordinated metabolic benefits across adipose and liver tissues [144].

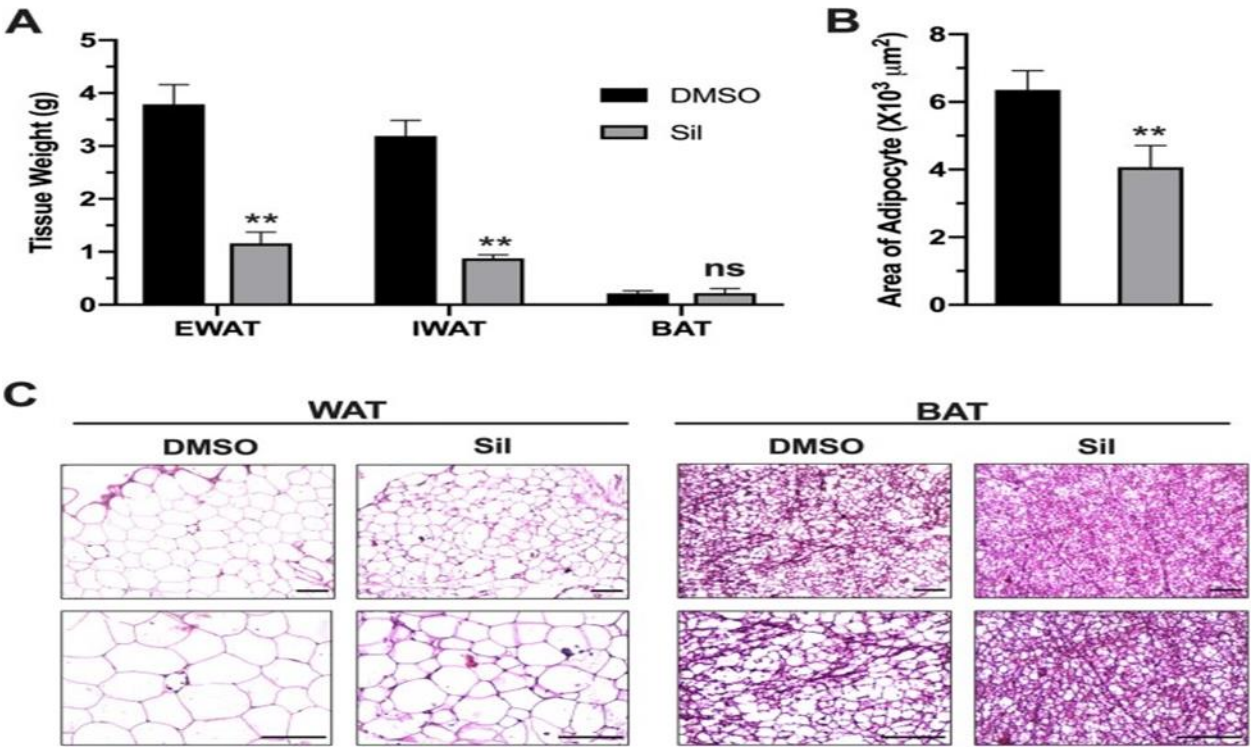

Figure 12 : Silibinin treatment suppressed adipose tissue hypertrophy. [140]

2.4. Silibin's anti-obesity and anti-diabetic properties

2.4.1 In vitro studies

In organs and biological systems that do not rely on insulin for glucose transport—such as the testis, placenta, peripheral and central nervous systems, ophthalmic lens, and pancreatic islets—intracellular sorbitol accumulation occurs under hyperglycemic conditions. This process is facilitated by the enzyme aldose reductase, leading to cellular water retention and subsequent tissue damage. Silibin, a primary active component of milk thistle, functions as an aldose reductase inhibitor, positioning it as a potential candidate for the prevention and treatment of diabetic complications such as cataracts and neuropathy. Notably, in an in vitro model using SY5Y neuroblastoma cells exposed to high glucose concentrations, treatment with 10 nM silibin prevented glucose-induced reductions in mono-ADP-ribosylation and preserved Na^+/K^+-ATPase activity. These protective effects were not observed with equimolar concentrations of fructose or galactose [[145], [146]].

In perifused rat hepatocytes incubated with dihydroxyacetone (DHA), silibin at concentrations of 25 μM and 100 μM was shown to reduce DHA-driven gluconeogenesis by

33% and 49%, respectively, while also suppressing glycolysis as evidenced by decreased lactate and pyruvate production. These metabolic shifts are attributed to a reduced NADH/NAD redox state and a lowered cytosolic ATP/ADP ratio, indicating diminished cellular oxygen consumption. Moreover, silibin variably inhibited glucose-6-phosphate hydrolysis regardless of the carbohydrate source, though it appeared to have negligible effects on the fructose cycle [147].

In cultured 3T3-L1 preadipocytes, silibin dose-dependently inhibited their differentiation into mature adipocytes, resulting in lower intracellular triglyceride accumulation. This inhibitory action is pronounced during the initial 48 hours of cell treatment. Mechanistically, silibin downregulated the expression of adipogenic genes including C/EBP, PPARα, aP2, FAS, LPL, and SREBP1c, while upregulating pref-1, a marker that is typically repressed during adipocyte maturation. Additionally, silibin enhanced the expression of insig-1 and insig-2, regulatory proteins that suppress SREBP-mediated gene transcription. Notably, insig-2 also indirectly inhibits PPARγ, a central regulator of adipogenesis [148].

In type 2 diabetes, pancreatic β-cell failure is exacerbated by the accumulation of cytotoxic aggregates of human islet amyloid polypeptide (hIAPP). Silibin has been shown to improve β-cell viability by inhibiting hIAPP fibrillation and oligomerization while reducing its cytotoxicity in a dose-dependent manner [149]. Furthermore, isosilibin A, a constituent of silymarin, was identified as the first flavonolignan to function as a PPARγ agonist. At a concentration of 30 μg/ml, it elicited a 19% activation in a PPARγ-dependent luciferase reporter assay, while other silymarin components, including silibin A/B, isosilibin B, silichristin, silidianin, and taxifolin, lacked such activity [150].

2.4.2 Animal ex vivo studies

Silibinin has been shown to reduce hepatic glycolysis by inhibiting pyruvate kinase (PK) activity and decreasing dihydroxyacetone (DHA) phosphorylation, a process attributed to lowered intracellular ATP levels. Notably, even at a low concentration of 10 μM, silibinin effectively suppresses reactive oxygen species (ROS) production associated with DHA metabolism, with its full antioxidant capacity observed at concentrations ranging from 25 μM to 100 μM [151]. While silibinin does not directly reduce blood glucose levels, it has demonstrated neuroprotective properties by preventing excessive protein mono-ADP-ribosylation and preserving substance P-like immunoreactivity and axonal transport in the sciatic nerves of alloxan-induced diabetic rats at concentrations as low as 1 μM, suggesting its potential in managing diabetic neuropathy [152].

In a separate study, silymarin administration over a 60-day period led to improved glycemic control in rats with streptozotocin (STZ)/nicotinamide-induced diabetes. The treatment was also associated with enhanced renal function, evidenced by significant reductions in serum creatinine, urine volume, and urinary albumin levels. Histopathological evaluations supported these findings: diabetic rats treated with a low dose of silymarin exhibited signs of tubular regeneration and moderate intertubular hemorrhage, while those

receiving a high dose showed well-preserved tubules, intact epithelial linings, and minimal erythrocyte infiltration [153].

2.4.3 Animal in vivo studies

In alloxan-induced diabetic rats, silibinin significantly lowered serum glucose levels and protected pancreatic tissue from lipid peroxidation, as indicated by decreased malondialdehyde (MDA) levels and preservation of glutathione content [154]. In a similar model, silymarin administration led to glucose normalization beginning in the first week of treatment and reaching control levels by the ninth week, while insulin levels returned to normal within just 7 days. Gene expression analysis via RT-PCR revealed comparable mRNA levels of insulin and Pdx1—an essential transcription factor for insulin gene regulation—between treated and healthy rats, accompanied by morphological restoration of the pancreatic islets of Langerhans [154].

In rats subjected to partial pancreatectomy, oral silibinin at 200 mg/kg continued to exert antihyperglycemic effects two months post-surgery. This improvement was linked to stimulated beta-cell neogenesis, as evidenced by increased expression of insulin and Nkx6.1, a transcription factor essential for beta-cell differentiation and maintenance [155] [156].

Silibinin also modulates endothelial function in diabetes by lowering levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase (eNOS). Remarkably, in db/db mice, silibinin reduced ADMA levels to below those found in non-diabetic controls (db/m) [157]. Additionally, in models of diabetic retinopathy, silibinin exhibited vasoprotective effects by reducing vascular leukostasis and retinal intercellular adhesion molecule-1 (ICAM-1) expression [158].

Enhanced delivery systems, such as silibinin-loaded nanoparticles, significantly improved hypoglycemic activity compared to standard formulations in STZ-induced diabetic mice. This nanoparticle form also normalized HbA1c, insulin, cholesterol, triglycerides, liver enzyme levels, and antioxidant enzyme activities—including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH)—bringing these markers closer to those observed in non-diabetic controls [159]. Another study confirmed silymarin's antioxidant role, demonstrating restoration of SOD, glutathione peroxidase (GPx), and CAT levels, highlighting its effectiveness as a free radical scavenger in preventing alloxan-induced pancreatic damage [160], [161]

In the context of non-alcoholic steatohepatitis (NASH)—a frequent comorbidity in type 2 diabetes—five weeks of silibinin treatment (200 mg/kg) yielded multiple benefits. These included reduced liver weight and liver index, improved insulin levels and HOMA-IR scores, decreased cholesterol and triglycerides, and lower oxidative stress markers like MDA and GSH. Histological analysis revealed that over 60% of treated rats had mild (grade 0 or 1) steatosis, in contrast to the NASH group where all animals exhibited moderate to severe steatosis (grade 2 or 3). Additionally, tumor necrosis factor-alpha (TNFα) levels were significantly reduced. However, this study found no significant improvement in liver enzyme levels or mitochondrial function, nor did it reverse elevated CYP2E1 or reduced PPARα

expression [162]. In contrast, another study under similar experimental conditions reported significant reductions in alanine transaminase (ALT) and aspartate transaminase (AST) [163].

Given that visceral obesity is a key contributor to insulin resistance and type 2 diabetes, silibinin treatment in high-fat diet-fed rats resulted in notable reductions in body weight, visceral and subcutaneous fat deposits, and the visceral fat-to-body weight ratio. It also improved insulin sensitivity, evidenced by reduced HOMA-IR scores and enhanced insulin tolerance test (ITT) performance compared to controls [164]. Interestingly, while 20 mg/kg/day of silymarin led to weight gain after 15 days, it significantly decreased blood glucose levels starting from day two in STZ-induced diabetic rats. This hypoglycemic effect persisted for two weeks and was occasionally accompanied by hypoglycemia in both diabetic and control groups [165]. Unlike some in vitro studies, no changes in basal insulin levels were observed, leading researchers to hypothesize that silymarin's glucose-lowering effect may stem from suppressed hepatic glucose output and/or increased peripheral glucose uptake in muscle and adipose tissues—though this remains unconfirmed in vitro [165], [166]. Additional theories, such as restoration of insulin sensitivity or reduced renal glucose reabsorption, have been proposed but lack definitive experimental support.

In Psammomys obesus, a rodent model of human obesity and diabetes, oral silibinin (100 mg/kg/day for 7 weeks) significantly improved lipid metabolism, reduced insulin resistance, restored antioxidant capacity, and partially reversed hepatic steatosis [167]. Furthermore, in models of diabetic neuropathy, silibinin treatment alleviated formalin-induced hyperalgesia, improved thermal pain response, and reversed motor nerve conduction deficits [167]

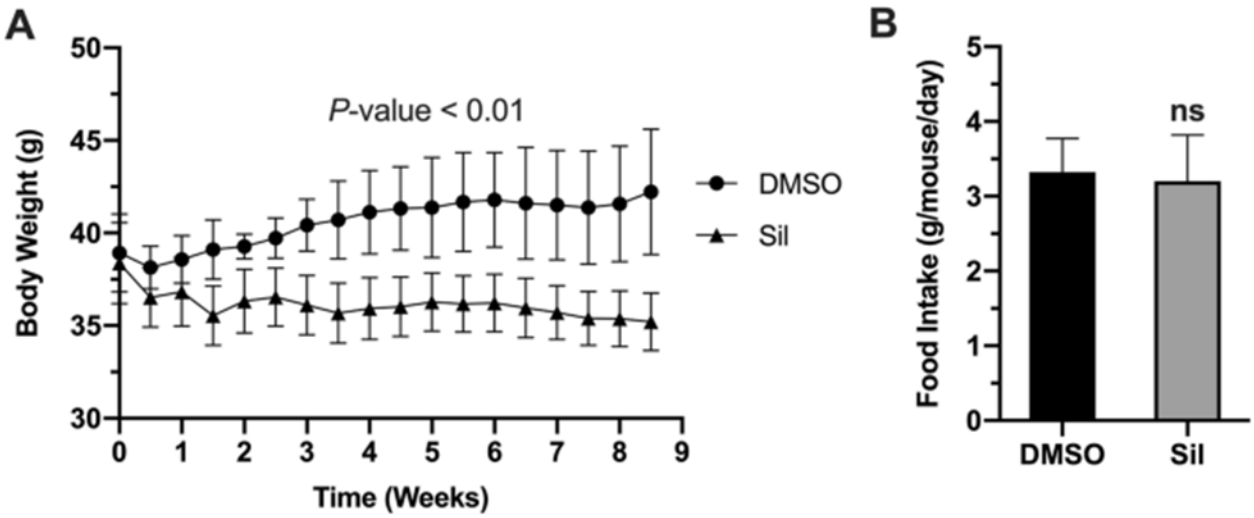

Figure 13 : Silibinin blocks progression of obesity [167].

3. Antidiabetic Effects of Milk Thistle: Protection of Pancreatic β-Cells:

3.1 . Mechanisms of β-Cell Protection:

3.1.1. Anti-Inflammatory Effects:

Silymarin inhibits cytokine-induced expression of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO), which are toxic to β-cells. It does this through inhibition of the NF-κB and MAPK signaling pathways [168].

3.1.2. Antioxidant Action

Oxidative stress plays a pivotal role in β-cell dysfunction. Silymarin enhances glutathione levels and reduces lipid peroxidation, thereby protecting β-cells from ROS-induced damage [169].

3.1.3. Regulation of Apoptotic Pathways

Silymarin modulates Bcl-2 and Bax protein expression, reducing β-cell apoptosis in experimental models. It stabilizes mitochondrial membranes and inhibits caspase activation [170]

3.2. In Vitro Evidence:

Studies using insulin-secreting cell lines like RINm5F and INS-1 show that silymarin reduces cytokine-induced NO production and cell death, while preserving insulin secretion under stress conditions [171].

3.3. In Vivo Animal Studies

In alloxan- and streptozotocin-induced diabetic rat models, milk thistle extract significantly restored pancreatic morphology, reduced fasting blood glucose, and improved insulin secretion. Upregulation of transcription factors like Nkx6.1 and Pdx-1 suggests β-cell regeneration [172] [173].

In alloxan- and streptozotocin-induced diabetic rat models, milk thistle extract significantly reduced fasting blood glucose levels, improved insulin secretion, and restored pancreatic morphology. These beneficial effects were linked to the upregulation of key transcription factors, including Nkx6.1 and Pdx-1, which are indicative of β-cell regeneration [174], [175].

4. Meta-Analytical Evaluation of Milk Thistle (Silybum marianum) in the Management of Type 2 Diabetes Mellitus: Therapeutic Efficacy and Mechanistic Insights

The increasing interest in botanical therapeutics for metabolic disorders has highlighted *Silybum marianum* (milk thistle) as a promising candidate for type 2 diabetes mellitus (T2DM), owing to its bioactive compound silymarin and its diverse pharmacological actions. This section presents a meta-analysis integrating preclinical and

clinical evidence to evaluate the antidiabetic efficacy of silymarin, focusing on its effects on glycemic parameters (FBG, HbA1c, insulin sensitivity), lipid profiles, and inflammatory markers. Mechanistic insights—such as modulation of insulin signaling pathways, suppression of hepatic gluconeogenesis, and antioxidant activity—are also discussed. The analysis aims to inform the potential role of milk thistle as an adjunctive therapy in T2DM, while identifying current evidence gaps and priorities for future clinical research.

4.1. Silymarin Inhibits Cytokine-Stimulated Pancreatic Beta Cells by Blocking the ERK1/2 Pathway:

These results showed that silymarin decreased iNOS gene expression, which is involved in pancreatic beta cell destruction.

Silymarin, a flavonolignan complex from Silybum marianum, was evaluated for its anti-inflammatory potential in pancreatic β-cells under cytokine-induced stress. Cytokines like IL-1β, TNF-α, and IFN-γ synergistically increase the expression of inducible nitric oxide synthase (iNOS), leading to cytotoxic nitric oxide (NO) production in β-cells. The study utilized MIN6N8a cells exposed to a cytokine mixture (CM) and demonstrated that silymarin significantly inhibited NO production in a dose-dependent manner, as shown in Figure. 14 [176].

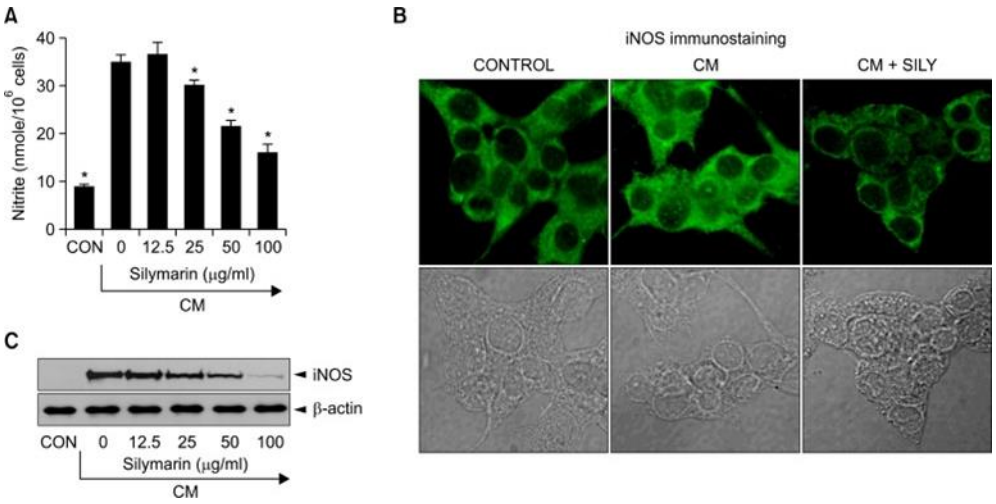

Figure 14: Effect of Silymarin on Nitric Oxide Production and iNOS Expression in CM-Stimulated Cells [176].

". In1hibition of the production of nitrite and iNOS by silymarin in cytokine-mixture (CM)-stimulated pancreatic beta cells. The pancreatic beta cell line, MIN6N8a, was treated with the indicated concentrations of Silymarin in the presence of cytokine mixture (CM: TNF-α, 500 U/ ml; IFN-γ, 100 U/ml; IL-1β, 10 U/ml) for 48 h. (A) Supernatants were subsequently

isolated and analyzed for nitrite. (B) MIN6N8a cells were treated with silymarin (50 μg/ml) in the presence of CM for 24 h on a cover slip in 12-well plates. Cells were subjected to immunofluorescence staining using an antibody specific for murine iNOS. The immunoreactive regions for iNOS were localized along the margins of the cytoplasm in the control group. (C) MIN6N8a cells were treated with the indicated concentrations of silymarin in the presence of CM for 24 h Expression of iNOS was analyzed by Western blot using an antibody specific for murine iNOS. Each column shows the mean ± S.D. of triplicate determinations. *$p<0.05$ compared to the control group as determined by Dunnett's two-tailed t test.

Reverse transcription PCR and Western blotting confirmed the transcriptional and translational suppression of iNOS. Figure 1B presented reduced iNOS mRNA expression, while Figure 1C confirmed suppression of iNOS protein in silymarin-treated groups [177]. These findings demonstrated that silymarin suppresses cytokine-induced inflammatory signaling at the gene expression level.

ERK1/2 phosphorylation, an upstream activator of inflammatory pathways, was markedly increased following cytokine stimulation. However, as shown in Figure 15 , silymarin pretreatment significantly attenuated this phosphorylation. The use of the MEK1 inhibitor PD98059, presented in Figure 2B, mirrored the suppressive effects of silymarin, thereby confirming that silymarin targets the MEK/ERK pathway to suppress iNOS induction [178].

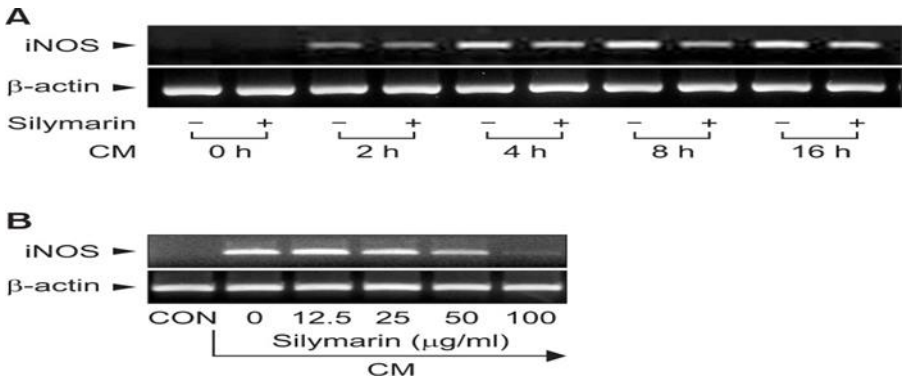

Figure 15: Effect of Silymarin on iNOS mRNA Expression in CM-Stimulated Cells [178].

"Inhibition of iNOS gene expression by silymarin in CM-stimulated MIN6N8a cells. (A) MIN6N8a cells were treated with silymarin (50 μg/ml) in the presence of CM for indicated times. (B) Cells were treated with indicated concentrations of silymarin in the presence of CM for 8 h. Total RNA was isolated and analyzed for mRNA expression levels of iNOS and β-actin."

Time-course analysis in Figure 16 revealed that ERK1/2 phosphorylation peaked at 30 minutes post-cytokine exposure and remained elevated for over 60 minutes. Silymarin

blunted this activation rapidly, starting as early as 15 minutes, suggesting an immediate upstream action in the inflammatory cascade [179].

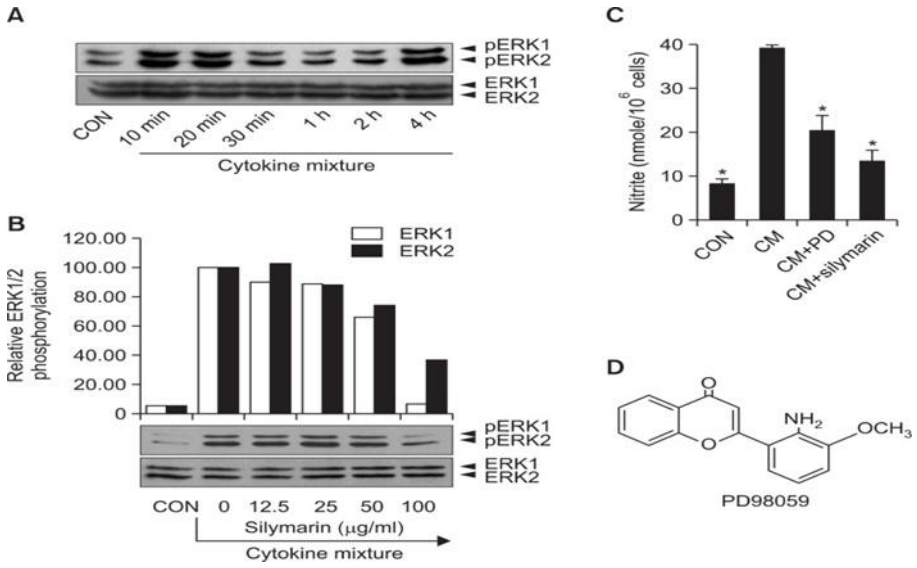

Figure 16: Inhibitory Effects of Silymarin on ERK1/2 Phosphorylation and Nitrite Production in CM-Stimulated Cells[179].

To examine downstream transcriptional regulators, the study investigated NF-κB activation. Cytokines induced nuclear translocation of the NF-κB p65 subunit, shown by increased nuclear p65 levels. Silymarin significantly suppressed this translocation., Western blotting of nuclear fractions supported this, confirming that silymarin hinders NF-κB activation, a central pathway in β-cell inflammation [180].

"Inhibition of p44/42 phosphorylation by silymarin in CM-stimulated MIN6N8a cells. (A) MIN6N8a cells were treated with CM for the indicated time. (B) Cells were treated with silymarin for 20 min in the presence of CM. The phosphorylation of p44/p42 was analyzed by Western blot. The relative band densities were analyzed with Image J program. (C) Cells were treated with PD98059 (50 μM) or silymarin (50 μg/ml) for 48 h in the presence of CM. The supernatants were subsequently isolated and analyzed for nitrite. Each column shows the mean ± S.D. of triplicate determinations. *$p<0.05$ compared to the control group as determined by Dunnett's two-tailed t test. (D) The chemical structure of PD98059."

While silymarin inhibited ERK1/2 signaling, it did not significantly affect other MAPKs like p38 or JNK (data not shown), indicating specificity toward the MEK/ERK cascade. This targeted action minimizes the risk of broadly inhibiting cellular signaling, an advantage over less selective kinase inhibitors [181].

Although not depicted in the figures, luciferase reporter assays showed that silymarin also suppressed NF-κB-dependent transcription, supporting its role in transcriptional inhibition of iNOS [182]. The compound's non-cytotoxicity was confirmed via MTT assay

(data not shown), proving that the observed effects were not due to general toxicity but rather pathway-specific modulation [183].

Hypoglycemic effect of silychristin A from Silybum marianum fruit via protecting pancreatic islet β cells from oxidative damage and inhibiting α-glucosidase activity in vitro and in rats with type 1 diabetes

Author links open overlay panelNingbo Qin a b, Xu Hu a b, Shengge Li a b, Jian Wang a, Zhanlin Li a b, Dahong Li a b, Fanxing Xu d, Ming Gao c, Huiming Hua a b

The graphical model in Figure 4 outlines the mechanism: cytokine receptors activate MEK, leading to ERK1/2 phosphorylation, NF-κB activation, iNOS expression, and NO release. Silymarin intervenes at the MEK/ERK step, reducing both ERK1/2 activation and NF-κB translocation, ultimately inhibiting NO production [184].

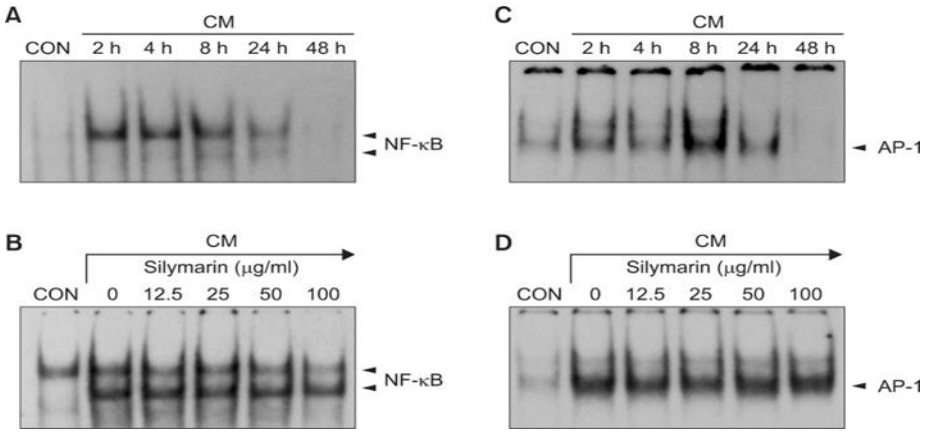

Figure 17: Effect of CM stimulation and silymarin treatment on NF-κB and AP-1 DNA-binding activity in cultured cells. [184].

"Inhibition of NF-κB activation by silymarin in CM-stimulated MIN6N8a cells. (A, C) MIN6N8a cells were treated with CM for the indicated times. Nuclear extracts were then isolated and analyzed for the activities of NF-κB (A) and AP-1 (C). (B, D) Cells were incubated with silymarin in the presence of CM for 2 h. Nuclear extracts were then isolated and analyzed for the activities of NF-κB (B) and AP-1 (D)."

These mechanistic insights suggest that silymarin may help preserve β-cell viability and function by blocking key inflammatory signals, potentially preventing cytokine-induced apoptosis in diabetes. While the study focused on type 1 diabetes models, similar cytokine profiles and oxidative stress mechanisms occur in type 2 diabetes, indicating a broader therapeutic relevance [185].

4.2. Hypoglycemic effect of silychristin A from Silybum marianum fruit via protecting pancreatic islet β cells from oxidative damage and inhibiting α-glucosidase activity in vitro and in rats with type 1 diabetes:

A recent study by Qin et al. Investigated the hypoglycemic effects of silychristin A, a flavonolignan compound derived from Silybum marianum (milk thistle) fruit, in both in vitro models and streptozotocin (STZ)-induced type 1 diabetic rats [186]. The authors demonstrated that silychristin A significantly reduced blood glucose levels in diabetic rats. This hypoglycemic activity was associated with preserved structural integrity of pancreatic islet β-cells and improved insulin secretion. In vitro, silychristin A was shown to suppress reactive oxygen species (ROS) accumulation and reduce β-cell apoptosis under conditions of STZ- or high glucose-induced oxidative stress [186].

Moreover, the study reported that silychristin A inhibited α-glucosidase activity, a key enzyme involved in carbohydrate digestion. This enzymatic inhibition may contribute to the reduction of postprandial glucose levels. Molecular docking analysis further supported this finding by revealing strong binding interactions between silychristin A and the active site of α-glucosidase, suggesting a potential mechanism for its inhibitory activity [186].

These findings suggest that silychristin A exhibits dual antidiabetic mechanisms by both protecting pancreatic β-cells from oxidative damage and modulating carbohydrate metabolism through α-glucosidase inhibition, making it a promising candidate for the development of novel antidiabetic therapeutics.

4.3. Antidiabetic Effects of Milk Thistle: Improves Insulin Sensitivity and Lowers Insulin Resistance:

Milk thistle (Silybum marianum), particularly its active compound silibinin (also known as silybin), has been extensively studied for its antidiabetic properties, notably its ability to enhance insulin sensitivity and reduce insulin resistance. Silibinin (also known as silybin), has been extensively investigated for its ability to modulate glucose metabolism. Research indicates that silibinin enhances insulin sensitivity by improving insulin receptor signaling pathways and reducing insulin resistance at the cellular level. These effects are attributed to its antioxidant, anti-inflammatory, and hepatoprotective actions, which collectively contribute to improved glycemic control in individuals with metabolic disorders, including type 2 diabetes mellitus."

4.3.1. Mechanisms of Action:

In individuals with type 2 diabetes mellitus (T2DM), the accumulation of human islet amyloid polypeptide (hIAPP) in pancreatic β-cells is a critical factor contributing to the progressive loss of insulin secretion capacity. Silibinin, a major bioactive component of silymarin, has demonstrated the ability to enhance β-cell survival by attenuating hIAPP aggregation. This compound inhibits fibril formation, promotes appropriate oligomerization, and decreases hIAPP-induced cytotoxicity in a dose-dependent manner [187], [188]. Furthermore, isosilybin A, another silymarin constituent, has recently been identified as the first flavonoglycan agonist of peroxisome proliferator-activated receptor gamma (PPARγ).

At a concentration of 30 μg/mL, it activated PPARγ by 19% in a luciferase reporter gene assay, while other flavonolignans such as silibinin A, silibinin B, isosilybin B, silychristin, silydianin, and taxifolin exhibited negligible activation [189].

• **PPARγ Activation:** Silibinin functions as a partial agonist of peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor involved in glucose metabolism. This activation can improve insulin sensitivity with potentially fewer side effects compared to full agonists like thiazolidinediones.

4.3.2. Therapeutic Potential of Milk Thistle in Modulating PPARγ Activity and Enhancing Insulin Sensitivity:

Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor that plays a central role in regulating insulin sensitivity and glucose homeostasis, making it a key therapeutic target in the management of type 2 diabetes mellitus (T2DM) [190]. Pharmacological activation of PPARγ by thiazolidinediones (TZDs), such as rosiglitazone and pioglitazone, was established even before their molecular mechanisms were fully elucidated [191]. The glucose-lowering efficacy of TZDs has been strongly associated with their ability to bind and activate PPARγ, a concept further supported by the insulin-sensitizing effects observed with non-TZD PPARγ agonists [192].

Genetic studies reinforce the essential role of PPARγ in metabolic regulation. Mice with a mutation that prevents phosphorylation at serine 112—leading to constitutively elevated PPARγ activity—are protected against obesity-induced insulin resistance [193]. Conversely, tissue-specific PPARγ knockout models in adipose tissue, skeletal muscle, and liver have shown disrupted insulin signaling and impaired glucose tolerance [194] [195] [196] [197]. In humans, rare dominant-negative mutations in the PPARγ gene cause severe insulin resistance [198], whereas the Pro12Ala polymorphism in PPARγ2 is linked to improved insulin sensitivity and glucose tolerance [199].

Recent research has turned attention to natural products, particularly bioactive compounds from Silybum marianum (milk thistle), for their potential role as modulators of PPARγ activity. Silymarin, a flavonolignan complex extracted from milk thistle, has demonstrated insulin-sensitizing properties that may involve PPARγ activation [200], [201]. Notably, silymarin and its constituents are being explored as phytotherapeutic alternatives to TZDs, potentially offering comparable metabolic benefits with fewer adverse effects [202].Collectively, these findings underscore the pivotal function of PPARγ in metabolic homeostasis and highlight milk thistle-derived compounds as promising adjuncts or alternatives in T2DM therapy, particularly through their potential to activate PPARγ pathways and enhance peripheral insulin responsiveness.

4.4. Silymarin as a Therapeutic Agent in Insulin Resistance and Metabolic Dysregulation

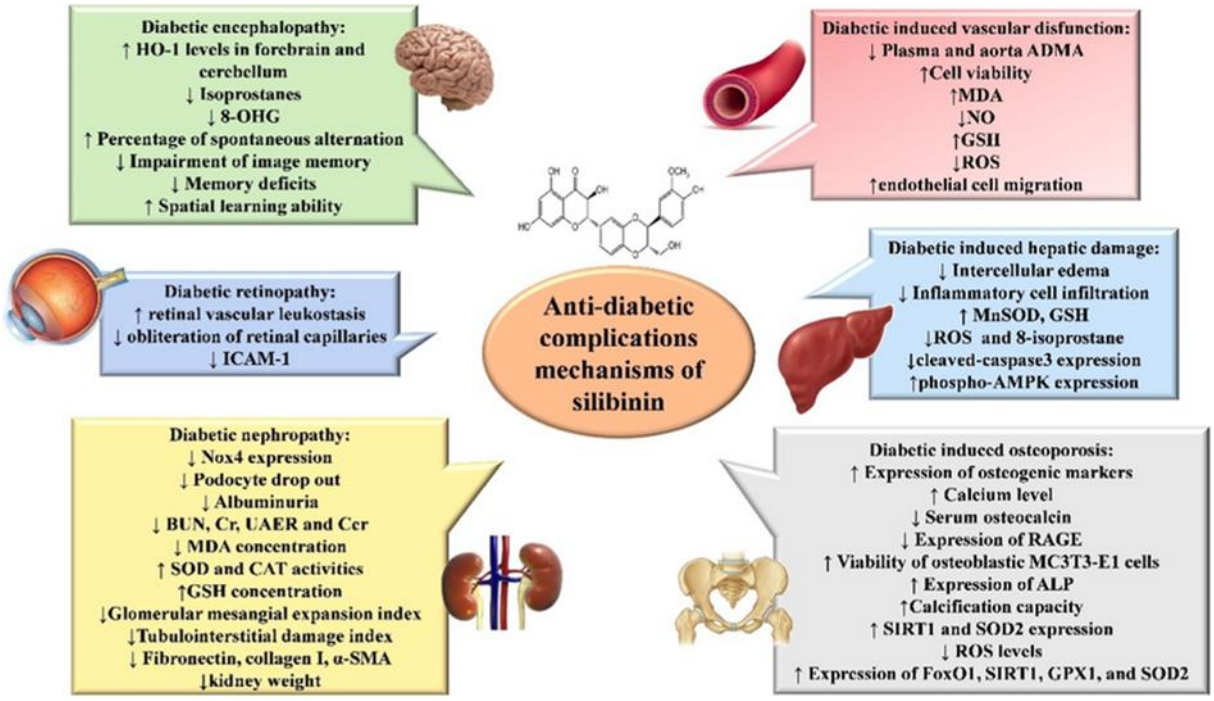

Figure 18: The protective effects of silibinin against DM complications [203].

4.4.1. Silymarin and Lipid Metabolism:

One of the earliest investigations into silymarin's lipid-lowering effects in animal models was conducted in 1998, using diet-induced hypercholesterolemic rats. The study reported that silymarin not only reduced hepatic cholesterol accumulation but also significantly elevated high-density lipoprotein (HDL) levels [203]. Given the observed synergistic benefits when silymarin is administered in combination with other supplements, subsequent studies have explored co-treatment approaches. For instance, the co-administration of silymarin and Prunella vulgaris in hypertriglyceridemic rats led to a reduction in plasma very-low-density lipoprotein (VLDL) levels and a favorable modulation of the plasma lipid profile [204]. In another experiment, a phytosomal formulation comprising silybin, phosphatidylcholine, and vitamin E was evaluated for enhanced bioavailability and lipophilicity. This complex, administered at 250 mg/kg, was tested in rat models of hepatic fibrosis induced by dimethylnitrosamine and bile duct ligation. The treatment attenuated liver injury, reduced hepatic stellate cell activation and proliferation, and inhibited collagen deposition [205]. Moreover, a comparative study assessed the cholesterol-lowering efficacy of whole silymarin versus its polyphenolic fraction in rats fed a high-cholesterol diet. Both interventions significantly decreased VLDL, total cholesterol, and hepatic triacylglycerol content. Notably, only the full silymarin extract enhanced HDL levels, whereas the polyphenolic fraction alone did not [206].

Non-alcoholic fatty liver disease (NAFLD), a global health concern increasingly linked to obesity and insulin resistance (IR), is associated with elevated risks of both hepatic and cardiovascular mortality [207]. NAFLD encompasses a spectrum of liver pathologies, ranging from simple steatosis to progressive liver damage, which may lead to hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [208]. A more severe manifestation, non-alcoholic steatohepatitis (NASH), represents an advanced inflammatory form of NAFLD [209]. Notably, environmental exposure to bisphenol A (BPA)—an endocrine disruptor with estrogen-like activity—has been implicated in NAFLD progression through mechanisms involving oxidative stress induction [210].

The therapeutic efficacy of silymarin, particularly silybin, has been explored in several animal models of NAFLD and NASH. In a rat NASH model, treatment with a silibinin–phosphatidylcholine complex (200 mg/kg/day) over five weeks significantly improved hepatic steatosis and inflammation, and decreased lipid peroxidation, plasma insulin levels, and tumor necrosis factor alpha (TNFα) [209]. In another NAFLD model, rats fed a high-fat diet (HFD) received silybin (26.25 mg/kg/day), resulting in lowered serum alanine aminotransferase (ALT), reduced hepatic malondialdehyde (MDA), increased adiponectin expression, and suppressed resistin expression. Additionally, silybin improved mitochondrial membrane fluidity [211].

Further studies reinforced these findings. In one model, silibinin (0.5 mg/kg/day) prevented visceral obesity and reduced visceral adiposity in HFD-fed rats with induced NAFLD. It also modulated lipid metabolism by upregulating adipose triglyceride lipase and downregulating key gluconeogenic enzymes such as Forkhead box O1 (FoxO1), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase) [212]. Another 12-week rat study employing silibinin (100 mg/kg/day) demonstrated reduced hepatic lipid accumulation, improved cell viability, and mitigated steatosis [213].

An experimental model using the gerbil Psammomys obesus examined the effects of silibinin (100 mg/kg/day) administered after seven weeks of a 14-week high-calorie diet. Silibinin significantly reduced triglyceride (TG) levels, improved hepatic metabolism, and partially reversed hepatic steatosis [214]. Another study evaluated three formulations of silymarin—standardized extract, micronized silymarin, and phytosome silymarin—in hereditary hypertriglyceridemic rats. All treatments significantly reduced plasma TG and total cholesterol (TC) levels while increasing high-density lipoprotein (HDL). Among them, micronized and phytosome formulations were more efficacious. The observed hypolipidemic effects may be partly mediated by upregulated protein expression of cytochrome P450 enzymes CYP7A1 and CYP4A [215].

Further studies supported these findings. In a high-cholesterol diet (HCD) model, silybin administered at 300 and 600 mg/kg dose-dependently reduced TC, TG, and low-density lipoprotein (LDL) levels while increasing hepatic HDL [216]. Similarly, in a murine NAFLD model, silymarin treatment attenuated hepatic steatosis, elevated HDL, andreduced LDL concentrations. Additionally, mRNA expression levels of lipid metabolism-related genes were modulated, although no significant changes in liver transaminases were observed [217].

Another mouse NAFLD model involved feeding animals a high-fat diet (HFD) for three months to induce insulin resistance and obesity, followed by supplementation with silymarin (40 mg/100 g diet) for six weeks. The intervention improved dyslipidemia and enhanced Farnesyl X receptor (FXR) transactivity, suggesting a mechanism through FXR signaling [218]. In vitro, HepG2 cells exposed to bisphenol A (BPA, 0.05 μM)—a compound known to promote oxidative stress and NAFLD progression—demonstrated reduced glucose uptake and lipid peroxidation upon treatment with silybin extract (68 μM). Silybin also activated vitamin D3 metabolite synthesis and prevented steroid hormone oxidation, indicating potential for reversing BPA-induced hepatic damage [219].

In a murine NASH model, where animals were fed a methionine–choline-deficient diet and co-treated with silibinin for six weeks, results showed significant activation of CFLAR (CASP8 and FADD-like apoptosis regulator), inhibition of c-Jun N-terminal kinase (JNK) phosphorylation, and reductions in alanine aminotransferase (ALT), aspartate aminotransferase (AST), hepatic TG, TC, and malondialdehyde (MDA). These effects were mediated by enhanced β-oxidation and fatty acid efflux, mitigating lipid accumulation in the liver [220].

Collectively, findings from these experimental models consistently demonstrate silymarin's ability to improve lipid metabolism and mitigate hepatic lipid accumulation. However, despite promising preclinical data, controlled clinical trials are essential to validate these effects in humans. Moreover, silymarin has shown significant benefits in carbohydrate metabolism, which will be discussed in the following sections.

4.4.2. Silymarin in Pancreatic Damage Recovery and Carbohydrate Metabolism:

One of the very first animal models to test silymarin's effects on carbohydrate metabolism was performed in rats treated with alloxan to induce T2D in the animals. Silymarin was then administered to the animals (200 mg/kg body weight) for up to seven days. Results demonstrated that silymarin was able to induce recovery of pancreatic function through the expression of insulin and glucagon proteins, normoglycemia, and recovered insulin serum levels [221]. Another study analyzed the effect of silymarin in the Pdx1 transcription factor, central to insulin gene expression, in partially pancreatectomized rats. Silymarin treatment induced an increase in both Pdx1 and insulin gene expression and hence, serum insulin levels rose. Results suggest that silymarin may induce the proliferation of insulin-producing cells [222]. In a similar partially pancreatectomized rat model, the effect of silymarin on the Nkx6.1 transcription factor (key for differentiation, neogenesis, and maintenance of β-pancreatic cells) was tested. Rats were treated with silymarin (200 mg/kg/day) for periods of up to 63 days. Silymarin-treated groups showed an increase of Nkx6.1 and insulin genic expression, β-cell neogenesis, and a rise in serum insulin and glucose levels [223].

As silymarin has been shown to have various degrees of bioavailability depending on the type of formulation used, a study tested a nanoparticle design in a diabetes animal model induced through streptozotocin. After 28 days of silymarin treatment in animals, blood glucose levels returned to near normal values and serum insulin was normalized. There was a significant reduction in glycated hemoglobin (HbA1c) levels and liver glycogen was restored [224]. Another diabetes model investigated the cytoprotective activity of silymarin against diabetes-induced cardiomyocyte apoptosis. After animals were treated with silymarin (120 mg/kg/day) for ten days, glucose levels returned to normal and exhibited pancreatic β-cell restoration [225]. Finally, in a study with hereditary hypertriglyceridemic rats, the micronized form of silybin significantly decreased glucose and insulin levels [226].

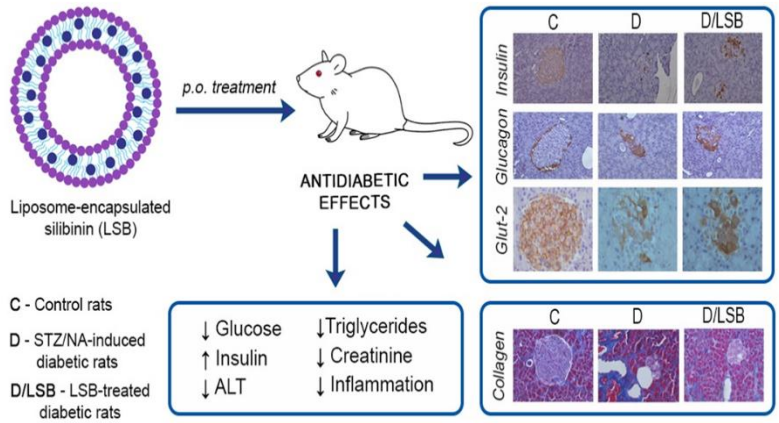

Figure 20: Antidiabetic Effects of Liposome-Encapsulated Silibinin (LSB) in STZ/NA-Induced Diabetic Rats [226].

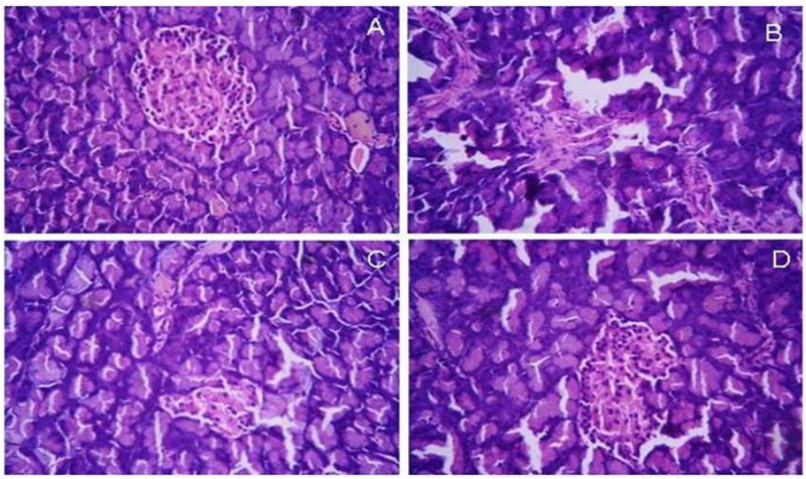

Figure 19: Histopathology examination of pancreas sections. (A) Normal Pancreas, (B) Diabetic Pancreas, (C) Sb treated pancreas, (C) CSbnp treated pancreas (Magnification 1006). Representative micrographs are shown. [226].

4.4.3. Silymarin ameliorates insulin resistance (IR):

Insulin resistance (IR) is intricately involved in various liver pathologies and is widely recognized as a precursor to type 2 diabetes mellitus (T2DM). It is particularly prevalent among individuals who are overweight or obese [227] In such metabolic states, IR is commonly accompanied by chronic low-grade inflammation. Obesity fosters a pro-inflammatory environment that is central to the initiation and persistence of IR [228]. Notably, studies have shown that reductions in tumor necrosis factor-alpha (TNF-α) levels within adipose tissue enhance insulin sensitivity in peripheral tissues. TNF-α contributes to IR by activating intracellular kinases such as c-Jun N-terminal kinase (JNK) and IκB kinase (IKK), which inhibit insulin receptor signaling through the phosphorylation of serine residues on insulin receptor substrate-1 (IRS-1) [229].

Moreover, the transcription factors Activator Protein 1 (AP-1) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), which are upregulated in obesity, promote the expression of pro-inflammatory cytokines. These cytokines are secreted into the bloodstream, resulting in elevated systemic inflammation that impairs insulin action in the liver and skeletal muscle [230]. Under non-insulin-resistant conditions, insulin binding to its receptor activates the IRS-1/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling cascade, which stimulates the translocation of glucose transporter type 4 (GLUT4) to the cell membrane, facilitating glucose uptake . This cascade also involves the recruitment of adaptor proteins to phosphorylated tyrosine residues on the insulin receptor, further propagating insulin signaling [231]

In the context of obesity, however, hyperglycemia, elevated free fatty acids, and increased levels of pro-inflammatory cytokines collectively elevate reactive oxygen species (ROS) and oxidative stress. These disturbances activate serine/threonine kinases such as JNK and IKK-β, contributing to the disruption of insulin signaling and the development of IR [232] [233] [234] [235] [236]. The following section presents studies examining the effects of silymarin on these pathological mechanisms, with a focus on its potential to alleviate IR.

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), plays a critical role in endothelial dysfunction, a common feature in insulin resistance (IR). The liver is central to regulating ADMA levels, and reductions in ADMA have been linked to improved IR [237]. In db/db mice, silibinin treatment improved endothelial function by lowering both circulating and vascular ADMA levels. In a non-alcoholic fatty liver disease (NAFLD) rat model, six weeks of high-fat diet (HFD) followed by silybin administration (26.25 mg/kg/day) significantly ameliorated IR [212]. A subsequent study using a similar model demonstrated that silibinin (0.5 mg/kg/day) improved glucose metabolism, as evidenced by reductions in the HOMA-IR index and enhanced insulin tolerance test (ITT) performance [213]. In another HFD-induced NAFLD rat model, silibinin (100 mg/kg/day) administered for 12 weeks alleviated IR by downregulating resistin and restoring IRS-1/PI3K/Akt signaling [214].

Conversely, some studies suggest a potential for silymarin to aggravate IR. In a fructose-rich diet model, rats treated with silymarin (200 mg/kg/day) showed increased

PTEN expression in skeletal muscle and liver, which negatively affected insulin signaling. This was corroborated in L6 myotube cells, where silymarin reduced Akt phosphorylation and induced a high-glucose environment [238]. However, in the Psammomys obesus gerbil model, silibinin (100 mg/kg/day) administered after seven weeks of a high-calorie diet resulted in reduced IR [215]. Similarly, in palmitate-treated C2C12 myotubes, silibinin alleviated IR by suppressing phosphorylation of JNK and IKKβ, thereby preserving the IRS-1/PI3K/Akt signaling pathway [239].

In a study involving obese mice, treatment with silymarin led to significant reductions in body weight and epididymal fat mass. Additionally, improvements in lipid profiles and a decrease in systemic inflammation were observed, indicated by reduced levels of pro-inflammatory cytokines, alleviation of hepatic histological damage, and enhancement of insulin sensitivity [240]. Another study using high-fat diet (HFD)-fed mice demonstrated that silymarin attenuated body weight gain, glucose intolerance, and IR by reducing oxidative stress markers, NF-κB expression, and TNFα concentrations in the liver [218]. Further evidence from an HFD-induced obesity model showed that silymarin (40 mg/100 g) modulated transcriptional activity of NF-κB and farnesoid X receptor (FXR), resulting in amelioration of IR [241]. In a separate experiment, administration of Silybum marianum extract (SME) to rats after 11 weeks on an HFD resulted in notable improvements in insulin sensitivity following 7 and 11 weeks of treatment [242].

Given these findings, elucidating the mechanisms by which silymarin improves hepatic function through reductions in IR may provide valuable insights into therapeutic approaches for liver-related metabolic disorders. The subsequent section focuses on

clinical trials that assessed not only metabolic parameters but also fasting or daily glucose, insulin levels, and HOMA-IR to evaluate the effects of silymarin on IR.

4.4.4 Silymarin in clinical trials:

In the latest clinical trials, silymarin therapy in patients with T2D, NAFLD, cirrhosis, and HCV has yielded encouraging outcomes regarding IR. One of the first clinical trials in human subjects with silymarin therapy for IR was to ascertain the impact of silymarin in 60 diabetic patients with cirrhosis. Patients were put on 600 mg silymarin per day (Legalon®) or placebo for six months, along with standard therapy. Mean fasting blood glucose (FBG), daily blood glucose (DBG), daily glycosuria, HbA1c, daily insulin need, and fasting blood insulin (FBI), among others, decreased in the silymarin-treated individuals. The observations suggest that in combination with exogenous insulin, silymarin possesses the capacity to improve the sensitivity of insulin receptors [243]. Several years later, the same research group conducted a 12-month open controlled trial in 60 diabetic patients with cirrhosis, treated with silymarin or placebo as in the previous study, and standard therapy. Reduction in FBI levels, mean DBG levels, daily glycosuria, and HbA1c was noted after four months of treatment. A reduction at the end of treatment was significant for FBI levels, mean requirements for exogenous insulin, basal, and glucagon-stimulated levels of C-peptide and MDA levels to evaluate liver fibrosis. Decrease in lipid peroxidation, IR, hyperproduction of endogenous insulin, and exogenous insulin requirement was facilitated through silymarin administration [244]. Similar to the patients undergoing treatment in these trials, a double-

blind, randomized placebo, three-center trial was performed in 42 diabetic patients with concomitant chronic liver disease. A silybin-beta-cyclodextrin (IBI/S) preparation was used (135 mg/day silybin orally) for six months. The treated patients showed a significant reduction in FBI and Tg values, and the same trend was observed for DBG, HbA1c, and HOMA-IR, though without statistical significance. Insulin release was not affected, similar to TC and HDL levels [245].

In a separate study, a different silybin complex with vitamin E and phospholipids (188 mg silybin daily, RealSIL®) or placebo was given to 85 patients with NAFLD with or without HCV (the latter non-responders to treatment with interferon and ribavirin) for six months. Carbohydrate metabolism parameters and liver fibrosis were analyzed and treatment was found to improve IR. Results showed a significant decrease in HOMA-IR and insulinemia, as well as ALT, Gamma-glutamyl transferase (GGT) levels and liver fibrosis in both groups [246] [247] [248]. In another study, silymarin was administered to 96 NAFLD patients and 32 healthy controls with either silymarin (600 mg daily) or GKY (traditional Chinese herbal mixture). Although there was no change in IR, there was a significant decrease in Tg, TC, and ALT levels in the NAFLD group treated with silymarin [249]. A randomized double-blind clinical trial in 51 diabetic patients received silymarin (600 mg daily) or placebo for four months, plus conventional therapy. Results showed a significant decrease in HbA1c, FBG, TC, LDL, HDL, Tg, ALT, and AST levels, but no significant decrease in insulin levels after treatment [250]. Another study used the same silymarin complex and dosage as Trappoliere et al. For three months in 30 patients with chronic HCV, and ten patients with hepatic steatosis but without HCV. After treatment, patients with HCV showed a significant decrease in ALT, AST, C-reactive protein (CRP), Interleukin-2 (IL-2) and Interleukin-6 (IL-6) but no significant decrease in HOMA-IR, insulinemia or serum glucose. However, patients without HCV showed a significant decrease in ALT, AST, GGT, and alkaline phosphatase, Tg, FBG, insulinemia, HOMA-IR, CRP, Interferon-gamma (IFN-ɣ), TNFα, and IL-6 levels, after treatment. Results suggest that silymarin was able to improve liver and carbohydrate metabolism in patients with NAFLD more effectively without HCV infection, than those with HCV infection [251]. Another study in 138 patients with NAFLD and 36 patients with HCV used the same silybin complex as in the previous study, or placebo for 12 months together with recommended lifestyle modifications and individually designed diets in a multicenter, phase III, double-blind clinical trial. After treatment, results showed significant improvements in liver enzyme plasma levels, HOMA-IR, and liver histology [252].

A different silymarin formulation combined with another nutraceutical was given to 22 diabetic patients with suboptimal glycemic control despite the use of standard therapy. The formulation consists of silymarin and berberine, the latter used to treat hypercholesterolemia and diabetes. Patients were given silymarin 210 mg (Berberol®) for 90 days. After treatment, levels of HbA1c, basal insulin, HOMA-IR, TC, LDL, and Tg levels were significantly reduced. Interestingly, no significant changes were seen in HDL, FBG, body mass index (BMI), weight, or waist circumference (WC) [253]. Berberol® was again used in another clinical trial with 105 overweight, euglycemic, dyslipidemic patients at low cardiovascular risk for three months in a double-blind, placebo-controlled design. Patients

first underwent a six-month run-in period with diet and physical activity recommendations. After treatment, patients underwent a two-month wash-out period followed by treatment for three additional months. Treatment improved IR, with a significant decrease in FPI and HOMA-IR. Moreover, TC, LDL, and Tg levels decreased and HDL levels increased [254]. In another trial by the same research group, Berberol® (silymarin 210 mg) was used once more to treat 137 euglycemic, dyslipidemic patients who did not tolerate high doses of statins in a double-blind, randomized, placebo-controlled clinical trial. Patients who were able to tolerate half the dose of statins began treatment with Berberol® (silymarin 210 mg) for six months and statins with an adequate diet and physical activity. Treatment reduced IR, FBG, FPI, and HOMA-IR [255], [256].

A combination of silymarin and berberine that was previously used was attempted this time in combination with fermented rice extract in another study. Silymarin (210 mg/day, Berberol® K) or placebo was given to 143 hypercholesterolemic overweight normotensive patients, together with a proper diet and exercise, in a three-month randomised, double-blind study. Results depicted a significant decrease in total cholesterol (TC), low-density lipoprotein (LDL), triglycerides (Tg), high-sensitivity C-reactive protein, TNFα, and IL-6 levels in treated patients, with no significant decrease in fasting blood glucose (FBG) or HOMA-IR [257]. One more study that used silymarin along with berberine alone was carried out in 136 obese patients with type 2 diabetes (T2D), insulin resistance (IR), and other altered glucose and lipid parameters. Patients were given 210 mg silymarin or placebo and followed a low-calorie diet for 52 weeks. Treatment reduced IR, HOMA-IR, HbA1c, TC, LDL, Tg, and uric acid levels, and WC, trunk and visceral fat, and systolic and diastolic blood pressure significantly at six and 12 months. Moreover, HDL levels were significantly increased after 12 months [258]. In another study, 420 mg silymarin (Livergol®) or placebo was given to 40 diabetic overweight or obese patients in addition to standard treatment for 45 days in a parallel, randomized, triple-blinded, placebo-controlled clinical trial. Treatment reduced IR, insulin, HOMA-IR, and the Quantitative Insulin Sensitivity Check Index (QUICKI), TC, Tg, and LDL levels, and elevated HDL levels significantly [259]. A recent study investigated the effect of the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene variant in NAFLD patients on response to treatment with 420 mg Eurosil 85® (approximately 60% silymarin and 30 IU vitamin E) for six months in 54 NAFLD patients. In none of the patients was there an improvement in IR. However, the patients who did not have the genetic variant experienced significant reductions in AST, ALT, and GGT. The variant patients experienced a significant increase in HOMA-IR and glucose. The results of this study show that individuals with this variant, which occurred in 49% of the Hispanic population, experienced an unfavorable response to treatment [260]. Whether silymarin's effects are also determined by dietary habits based on genetic background is essential to determine [261].

Later, a recent randomized trial in 90 patients with NAFLD and 30 controls (healthy patients diagnosed with reflux disease) evaluated a new product compared to RealSIL®, vitamin D, RealSIL 100D® (303 mg of silymarin, vitamin E (used as a stabilizer of the formula), and vitamin D in a silybin–phospholipid complex) twice a day for six months with a follow-up after 12 months. Better values (normalization of the monitored variable into the

top level value of normal range) in IR via the reduction of insulin and HOMA-IR, together with steatosis were reported in treated patients [262]. Later, the same formula (RealSIL 100D ®) and dose as in the initial research were studied in another clinical trial on further 32 male NAFLD patients under treatment with BPA. Treatment decreased IR by decreasing insulinemia and HOMA-IR levels, as well as a variety of other metabolic indices, including ALT and AST, C-reactive protein, and TNFα. Interestingly, treatment with this silymarin preparation also decreased the concentrations of thiobarbituric acid reactive substances, lipid peroxidation markers, and oxidative stress in direct proportion to BPA exposure. Furthermore, treatment reduced elevated conjugated BPA and lowered its free form in urine. The results suggest that treatment may play a role in improved BPA detoxification, increase cellular antioxidant capacity in NAFLD patients, and importantly, reduce IR [263].

4.4.5. Antioxidant and Anti-inflammatory Effects:

Silymarin, a bioactive flavonolignan complex derived from Silybum marianum (milk thistle), exhibits potent antioxidant and anti-inflammatory properties, which are crucial for its therapeutic potential in metabolic disorders such as type 2 diabetes. It enhances the body's endogenous antioxidant defense mechanisms by increasing the activity and expression of enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are essential for neutralizing reactive oxygen species (ROS) and protecting cellular membranes from lipid peroxidation [264] [265] [266],. Moreover, silymarin elevates intracellular levels of reduced glutathione (GSH), a vital antioxidant involved in maintaining redox balance and supporting cellular detoxification [267] [268] [269]

In addition to its antioxidant activity, silymarin exerts substantial anti-inflammatory effects by modulating key inflammatory signaling pathways. It inhibits the activation of nuclear factor-kappa B (NF-κB), a transcription factor central to the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [268], [270]. By downregulating these mediators, silymarin reduces systemic inflammation and prevents cytokine-induced damage to pancreatic β-cells, a mechanism that contributes significantly to insulin resistance and the progression of type 2 diabetes.

Overall, the dual antioxidant and anti-inflammatory actions of silymarin enhance insulin receptor sensitivity and glucose metabolism. By mitigating oxidative stress and inflammation, silymarin helps restore insulin signaling pathways and holds promise for managing insulin resistance and associated metabolic conditions [271].

PART II: CHAPTER 03: MATERIALS AND METHODS

1.Materials and Methods

To explore the perceptions and practical use of Silybum marianum (milk thistle) in the context of diabetes management, a structured and validated survey questionnaire was designed and distributed to two target populations: individuals diagnosed with diabetes and healthcare professionals involved in their care. The instrument comprised both closed- and open-ended questions addressing participants' knowledge of milk thistle, patterns of use (including dosage forms and frequency), perceived therapeutic benefits, and any experienced or observed adverse effects. Ethical approval was obtained before data collection, and participation was voluntary and anonymous. The collected data were intended to provide complementary insights to existing pharmacological and clinical evidence, thereby contributing to a more comprehensive understanding of milk thistle's potential as an adjunctive treatment in diabetes care.

1.1. Survey Among Diabetic Patients

To gain insight into the knowledge, attitudes, and practices of diabetic individuals regarding the use of milk thistle (*Silybum marianum*) as a complementary remedy, a survey was conducted among elderly diabetic patients. The objective was to evaluate their awareness of this medicinal plant, actual usage, sources of recommendation, and willingness to consider its use within a clinical context.

The responses were compiled and analyzed, and the results are summarized in the following tables:

Table5: percentage of Prevalence of Awareness Regarding Milk Thistle (Silybum marianum) Among Respondents

Q1 – Have you ever heard of milk thistle (Silybum marianum)?		
	Frequency	Percent
Yes	76	76.0
No	24	24.0
Total	100	100.0

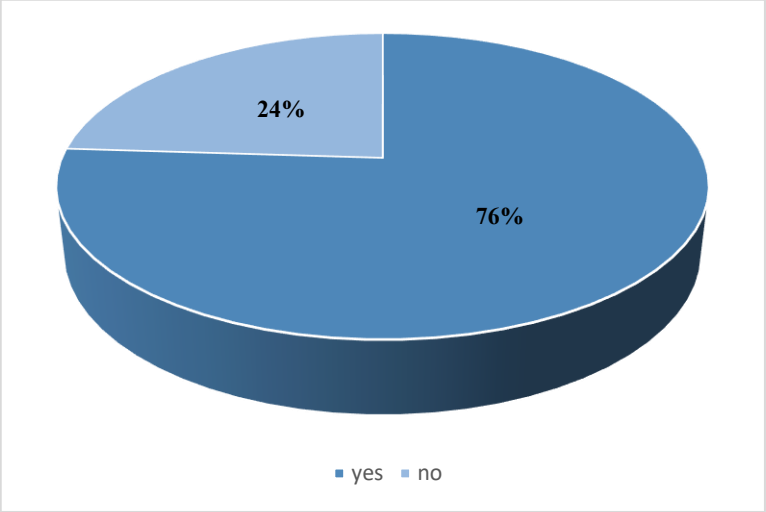

Figure 21: A proportional circle representing Prevalence of Awareness Regarding Milk Thistle (Silybum marianum) Among Respondents

Out of 100 respondents, 76% reported being aware of milk thistle, while 24% indicated they were not. This suggests a relatively high level of awareness of milk thistle among the surveyed population. The data may reflect either increased interest in or exposure to medicinal plants, possibly due to traditional knowledge or recent public health promotion. However, the 24% unfamiliarity also points to a need for broader education on the medicinal potential of such plants.

Table 6: percentage of Distribution of Milk Thistle Usage for Diabetes Management Among Respondents

Q2 – Have you ever used milk thistle for diabetes management?		
	Frequency	Percent
Yes	3	3.0
No	97	97.0
Total	100	100.0

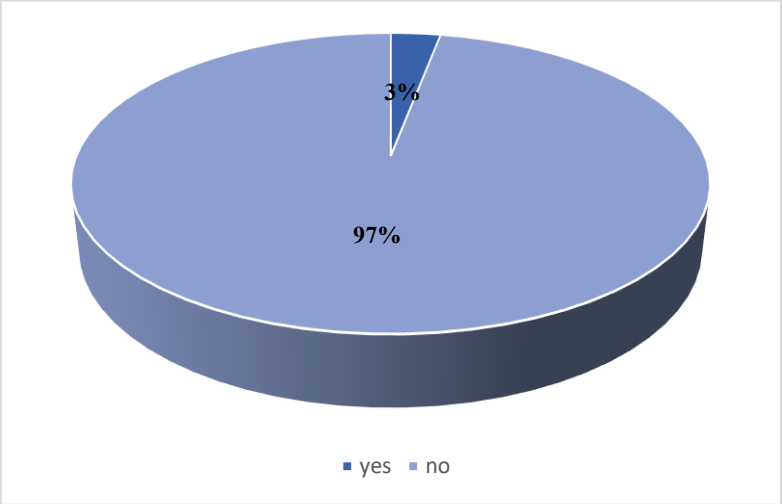

Figure 22: A proportional circle representing Distribution of Milk Thistle Usage for Diabetes Management Among Respondents

Only 3% of the participants reported having used milk thistle, whereas a striking 97% had not. Despite a majority claiming awareness of the plant (Q1), actual usage remains extremely low. This disparity suggests that awareness does not necessarily translate into use, possibly due to lack of access, skepticism regarding its efficacy, or preference for conventional medicine.

Table 7: percentage of Sources of Recommendation for Milk Thistle Use in Diabetes Management

Q3 – If yes, who recommended it to you?		
	Frequency	Percent
Herbalist	1	1.0
friend or family	1	1.0
internet or social media	1	1.0
no comment	97	97.0
Total	100	100.0

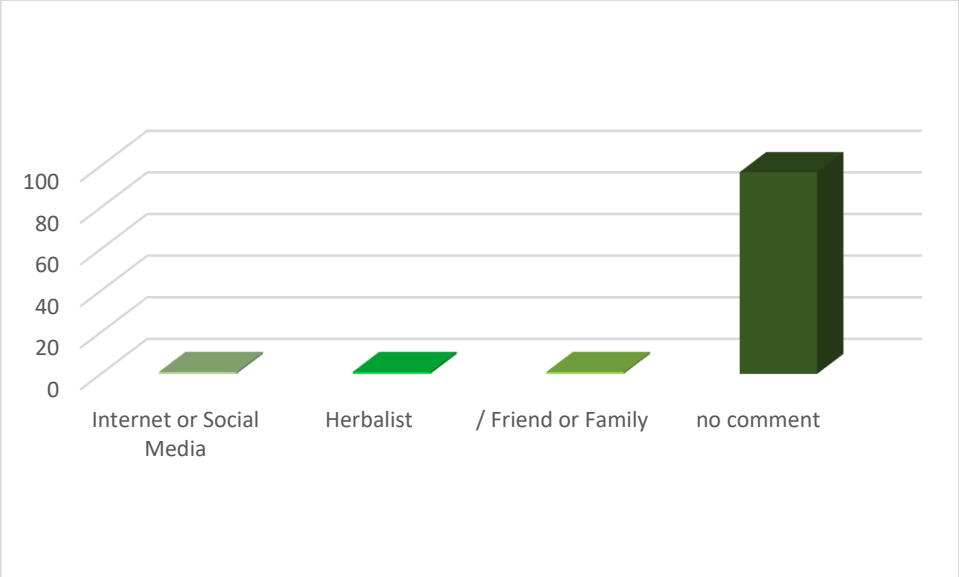

Figure 23: Bar graphs describe Sources of Recommendation for Milk Thistle Use in Diabetes Management

The responses were evenly distributed among three sources—herbalists, family/friends, and the internet/social media—with each accounting for 1%. However, 97% did not comment, likely correlating with the low number of actual users identified in Q2. This minimal feedback highlights the limitations in tracing the dissemination channels of herbal knowledge, and suggests the need for more targeted public education campaigns.

Table 8 : percentage of Duration of Milk Thistle Consumption Among Users for

Q4 – If you used milk thistle, how long have you been taking it?		
	Frequency	Percent
Less than one month	2	2.0
1-3 months	1	1.0
no comment	97	97.0
Total	100	100.0

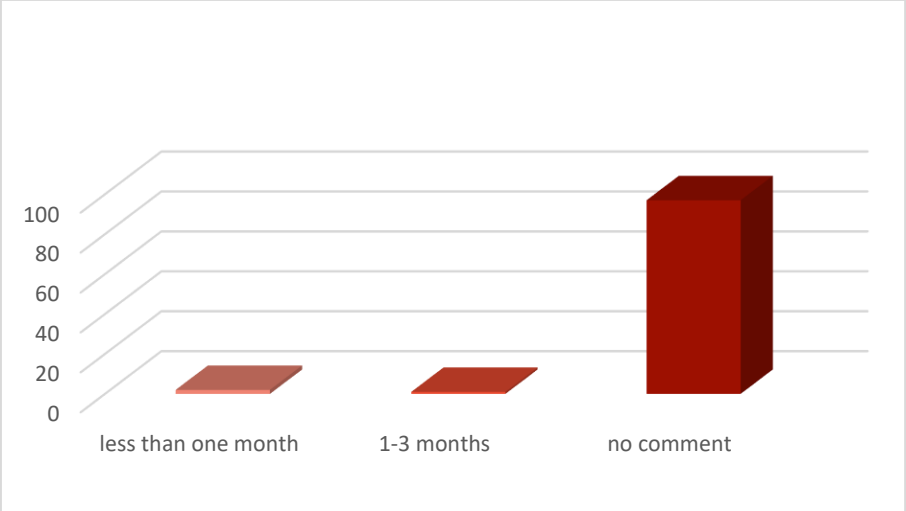

Figure 24: Bar graphs describe Duration of Milk Thistle Consumption Among Users for Diabetes Management (in Months/Years)

Diabetes Management (in Months/Years)

Only 3 participants provided responses, with 2% using it for less than a month and 1% for 1–3 months. This extremely limited usage duration further confirms that even among those who have tried milk thistle, sustained use is uncommon. The short duration could be due to lack of perceived benefits, side effects, or other personal factors.

Table 9: percentage of Reported Benefits in Blood Sugar Levels Following Milk Thistle Use

Q5 – Have you noticed any benefits in your blood sugar levels after taking milk thistle?		
	Frequency	Percent
Yes	3	3.0
no comment	97	97.0
Total	100	100.0

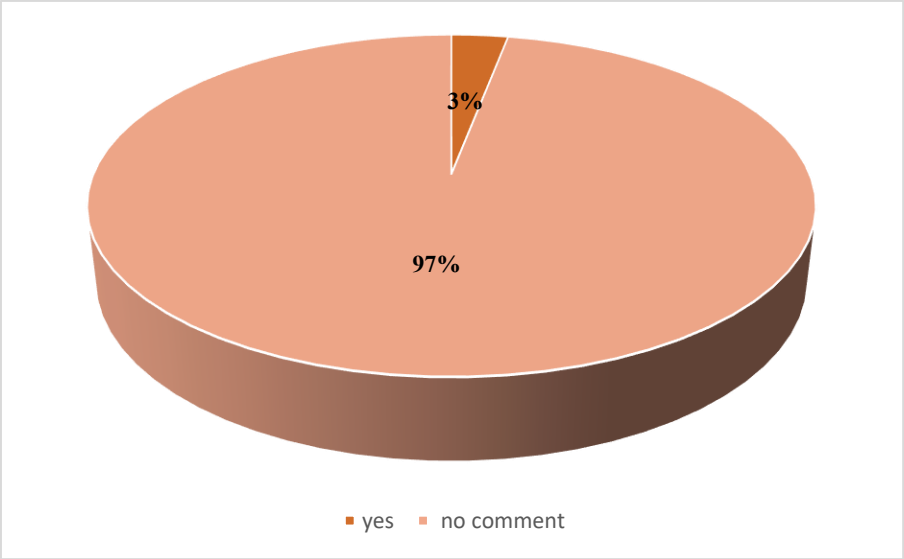

Figure 25 : A proportional circle representing Reported Benefits in Blood Sugar Levels Following Milk Thistle Use

Again, only 3% answered this question affirmatively, reflecting the same users from previous questions. This indicates that among the very few who used milk thistle, all perceived some level of improvement. While this finding is promising, the sample size is too small to draw definitive conclusions.

Table 10: percentage of Incidence of Side Effects Associated with Milk Thistle Consumption

Q6 – Have you experienced any side effects from milk thistle?		
	Frequency	Percent
No	3	3.0
no comment	97	97.0
Total	100	100.0

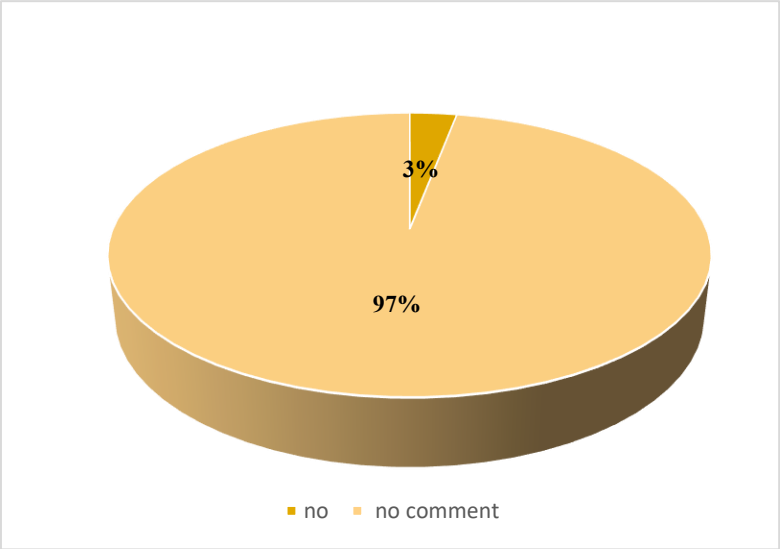

Figure 26: A proportional circle representing Incidence of Side Effects Associated with Milk Thistle Consumption

All 3 respondents reported no side effects, indicating a favorable safety profile based on this very limited data. Although this supports the generally accepted safety of milk thistle, broader studies are required to confirm its tolerability across diverse populations.

Table 11: percentage of Current Usage of Prescribed Diabetes Medications Among Respondents

Q7 – Are you currently taking any prescribed diabetes medications?		
	Frequency	Percent
Yes	94	94.0
No	6	6.0
Total	100	100.0

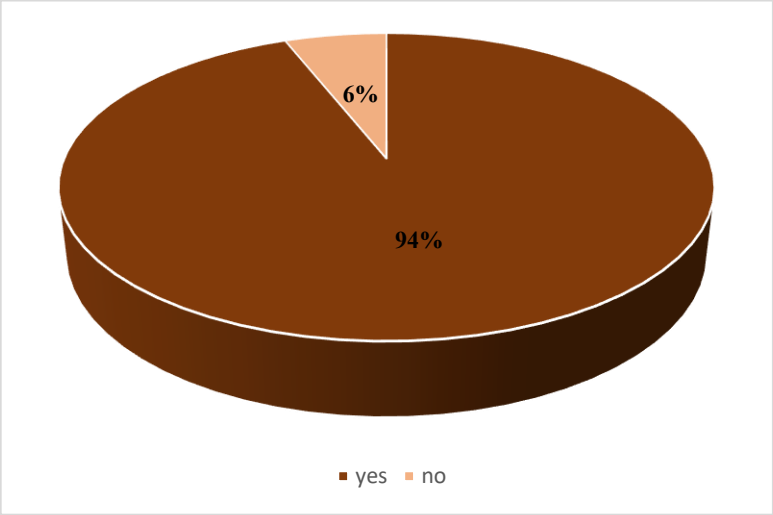

Figure 27: A proportional circle representing Current Usage of Prescribed Diabetes Medications Among Respondents

According to the results, 6% of participants reported that they are not currently taking any prescribed diabetes medications, while only 94% confirmed that they are. This distribution suggests that the vast majority of respondents are either non-diabetic or possibly managing their condition through non-pharmaceutical means such as lifestyle changes or traditional remedies. The very low percentage of individuals on prescribed medication may also reflect limited access to healthcare services, underdiagnosis, or a preference for alternative treatments in this population.

Table 12 : percentage of Proportion of Milk Thistle Users Who Consulted a Doctor Prior to Use

Q8 – Did you consult your doctor before using milk thistle?		
	Frequency	Percent
No	3	3.0
no comment	97	97.0
Total	100	100.0

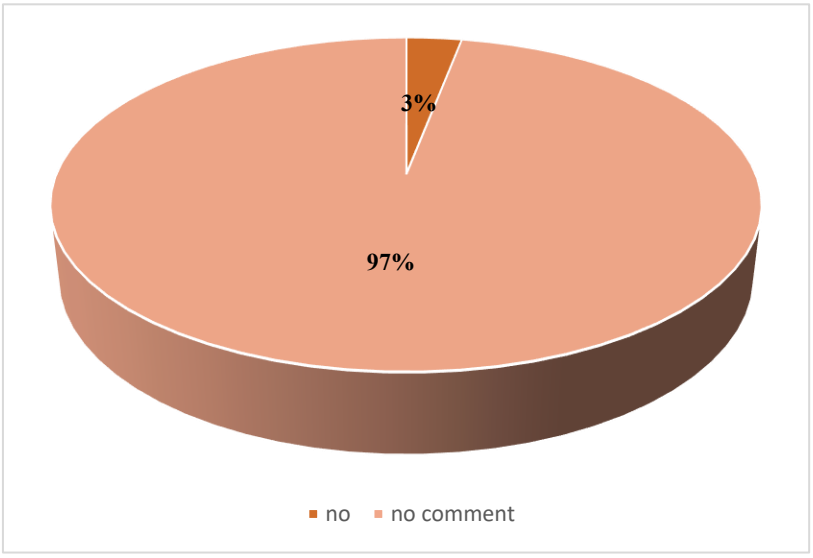

Figure 28: A proportional circle representing Proportion of Milk Thistle Users Who Consulted a Doctor Prior to Use

Only 3% of respondents said they had consulted a specialist prior to using medicinal plants. This indicates a concerning trend of self-medication without professional guidance, raising potential safety and efficacy issues. It underscores the necessity for better integration of traditional medicine practices within formal healthcare systems.

Table 13: percentage of Respondent Inclination Towards Future Milk Thistle Use Based on Professional Recommendation

Q9 – Would you consider continuing or starting milk thistle if recommended by a doctor or herbalist?		
	Frequency	Percent
Yes	79	79.0
No	21	21.0
Total	100	100.0

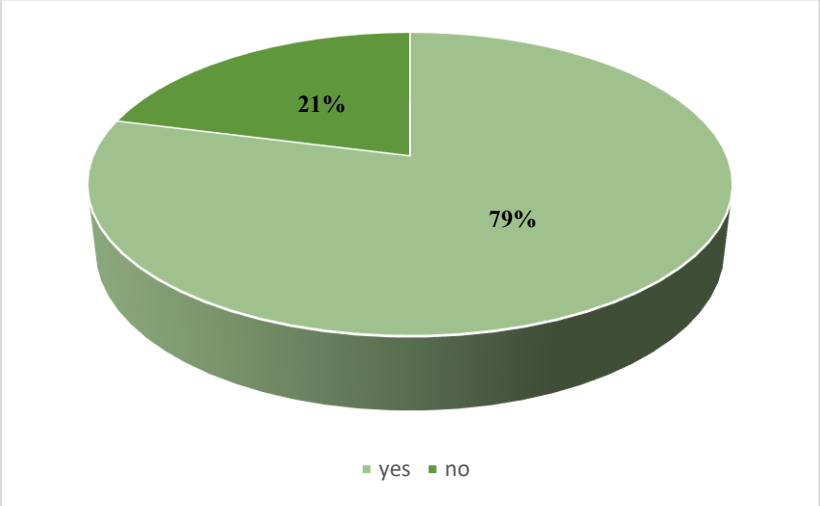

Figure 29 : A proportional circle representing Respondent Inclination Towards Future Milk Thistle Use Based on Professional Recommendation

A significant majority (79%) showed willingness to use milk thistle if its efficacy in managing diabetes is scientifically validated. This openness highlights the public's receptiveness to evidence-based herbal interventions and indicates potential for high acceptance if proper clinical validation and awareness campaigns are conducted.

Q10: **What other natural or herbal remedies have you tried for diabetes?**

The responses to Q10 revealed that the majority of participants reported using traditional herbal remedies such as **cinnamon, artemisia, fenugreek, and olive leaves** to manage diabetes. This reflects a strong cultural reliance on accessible, plant-based treatments, likely passed down through community and family traditions. The widespread use of these herbs highlights a perceived effectiveness among users, emphasizing the need for further scientific validation. These findings point to the importance of integrating traditional knowledge into clinical research while ensuring public education on the safe use of such remedies alongside conventional treatments.

1.2. Survey Among Healthcare Professionals

In order to assess the medical perception of milk thistle (*Silybum marianum*) in the context of diabetes management, a survey was conducted involving ten physicians. Among them, five were diabetes specialists (endocrinologists), and the remaining five were general practitioners. The aim of the questionnaire was to evaluate their level of awareness, clinical experience, and professional opinions regarding the potential use of milk thistle in diabetic patients.

The participants were asked the following ten questions:

1. **Are you familiar with milk thistle (*Silybum marianum*) and its potential health effects?** (Answer: Yes / No)
2. **Have you ever recommended milk thistle to diabetic patients?** (Answer: Yes / No)
3. **If not, what are the main reasons for not recommending it?** (Open-ended response)
4. **Are there any clinical trials or scientific evidence you rely on regarding the effects of milk thistle on diabetes?** (Answer: Yes / No – If yes, please specify)
5. **Do you believe that milk thistle plays an important role in blood glucose regulation?** (Answer: Yes / No / Uncertain)
6. **Do you consider milk thistle supplementation to be safe for diabetic patients?** (Answer: Yes / No / Under certain conditions – please explain)
7. **What are the potential risks or contraindications of using milk thistle in diabetic patients?** (Open-ended response)

8. **Have any of your patients reported using milk thistle without your recommendation?** (Answer: Yes / No)
9. **If yes, did they report any benefits or side effects?** (Open-ended response)
10. **Would you consider recommending milk thistle if more scientific evidence supported its benefits?** (Answer: Yes / No / Maybe)

The responses were compiled and analyzed, and the results are summarized in the following table:

Table 14: Summary of Physicians' Responses to the Milk Thistle (Silybum marianum) Questionnaire

Q / D	Q01		Q02		Q04		Q05			Q06			Q08		Q10		
	yes	no	Yes	no	Yes	no	yes	no	Not sure	yes	no	Under conditions	yes	no	yes	no	maybe
D01	Yes		no		no		Not sure			Under conditions			no		maybe		
D02	Yes		no		no		Not sure			Under conditions			no		maybe		
D03	Yes		no		no		Not sure			Under conditions			no		maybe		
D04	No		no		no		No			no			no		no		
D05	No		no		no		No			no			no		no		
D06	Yes		no		no		Not sure			Under conditions			no		maybe		
D07	Yes		no		no		Not sure			Under conditions			no		maybe		
D08	No		no		no		Not sure			Under conditions			no		maybe		
D09	No		no		no		Not sure			Under conditions			no		maybe		
D10	No		no		no		Not sure			no			no		no		

The responses to the open-ended questions (Questions 3, 7, and 9) were analyzed and summarized as follows:

- **Question 3** *(If not, what are the main reasons for not recommending it?)*: The most common reasons given were the lack of sufficient scientific evidence, absence of formal guidelines or recommendations, concerns about possible herb-drug interactions, and limited familiarity with the therapeutic effects of *Silybum marianum* in diabetes management.
- **Question 7** *(What are the potential risks or contraindications of using milk thistle in diabetic patients?)*: Reported risks included potential hypoglycemia when used alongside antidiabetic medication, allergic reactions, gastrointestinal discomfort, and uncertainties regarding long-term safety and dosage standardization.
- **Question 9** *(If yes, did they report any benefits or side effects?)*: none of them answered since all the repones were no

The chart illustrates the distribution of responses from ten surveyed physicians (five diabetes specialists and five general practitioners) to selected questions concerning the use of milk thistle (*Silybum marianum*) in diabetic care

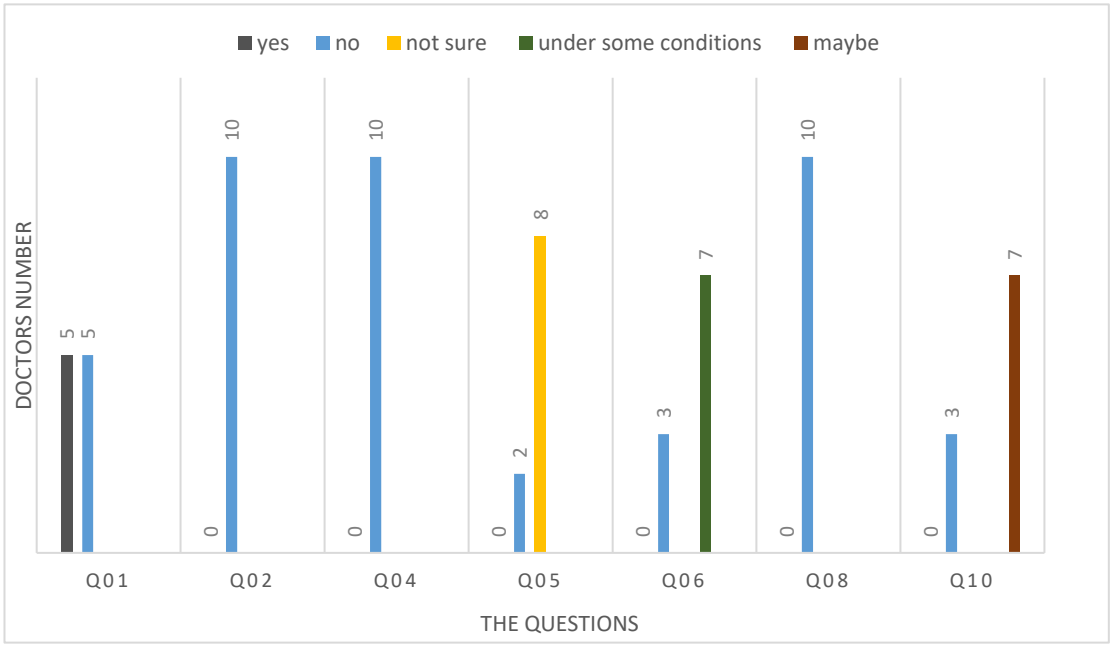

Figure 30: a chart that describe the Summary of Physicians' Responses Regarding the Use of Milk Thistle in Diabetes Management

1.2.1. Physicians' Responses Regarding Milk Thistle (*Silybum marianum*) and Diabetes Management

The chart summarizes the responses of ten physicians—five specialists in diabetology and five general practitioners—to a set of questions evaluating their knowledge, perceptions, and clinical attitudes toward the use of milk thistle (*Silybum marianum*) in diabetic patients.

- **Awareness (Q1):** Half of the respondents (5 out of 10) indicated they were familiar with milk thistle and its potential health benefits, suggesting a moderate level of awareness among physicians.
- **Clinical Recommendation (Q2):** None of the surveyed physicians had recommended milk thistle to their diabetic patients. This reflects either skepticism, lack of evidence, or insufficient familiarity with its clinical application.
- **Scientific Evidence (Q4):** Similarly, all ten physicians reported that they do not rely on any clinical trials or scientific data regarding milk thistle in diabetes, underlining a significant gap in accessible or trusted research on this subject in medical practice.
- **Perceived Efficacy (Q5):** When asked whether they believe milk thistle plays a significant role in blood glucose regulation, 80% of respondents answered "not sure", while only 2 were inclined to say "yes." This demonstrates widespread uncertainty about its effectiveness in glycemic control.
- **Safety Concerns (Q6):** Most respondents (7 out of 10) considered the use of milk thistle to be safe under certain conditions, such as medical supervision or depending on the patient's overall treatment plan. The remaining 3 physicians did not consider it safe, indicating caution and the need for clear clinical guidelines.
- **Patient-Initiated Use (Q8):** All physicians reported that none of their patients had informed them of using milk thistle without recommendation. This may indicate limited public use or lack of communication between patients and providers about herbal supplementation.
- **Future Recommendation (Q10):** Interestingly, despite their current hesitation, 7 physicians expressed that they might consider recommending milk thistle if further scientific evidence were available, indicating an openness to integrating phytotherapy in diabetes care—provided it is supported by rigorous research.

1.3. Data Analysis

Collected data were entered into **Microsoft Excel / SPSS** and analyzed using descriptive statistics (percentages, means, and standard deviations). The relationships between muscle-related symptoms and diabetes control variables (e.g., HbA1c, disease duration) were examined.

Results were presented in tables and graphics, and discussed in relation to existing literature in the next chapter

PART III: CHAPTER 04 : RESULTS AND DISCUSSION

1. Results:

This section will delve into a comprehensive analysis of the data collected from the structured questionnaires administered to elderly individuals with diabetes. It will explore in detail the levels of awareness surrounding milk thistle (*Silybum marianum*) and other prevalent herbal remedies, examining the patterns of their usage, the perceived benefits and drawbacks reported by users, and the sources of information influencing their choices. Furthermore, the discussion will address the participants' perceptions regarding the efficacy and trustworthiness of both prescribed diabetes medications and natural alternatives.

Key results pertaining to the prevalence of milk thistle awareness, the frequency and duration of its use, reported effects on blood sugar levels, any experienced side effects, and the extent of consultation with healthcare professionals will be presented and interpreted. The analysis will also shed light on the adoption of other herbal remedies for diabetes management and the reasons behind their selection.

Crucially, this section will discuss the interplay between the use of traditional medicine and conventional diabetes care among this demographic. It will highlight the degree of reliance on each approach, identify potential areas of overlap or conflict, and explore the factors influencing the decision to use herbal remedies, such as recommendations from family, friends, or alternative practitioners.

2. Discussion of Findings and Key Results :

2.1 Perspectives from Diabetic Patients

Based on the analysis of the questionnaire responses from elderly individuals living with diabetes, a general conclusion can be drawn regarding their awareness, use, and perception of milk thistle (*Silybum marianum*) and other herbal remedies. The findings are summarized as follows:

- **Limited Awareness and Use of Milk Thistle:** A significant portion of participants had never heard of milk thistle, indicating a low level of awareness of this particular herbal remedy among the elderly diabetic population.
- **Very Few Users of Milk Thistle:** Only a small number of participants had used milk thistle for diabetes management. Among them, most reported learning about it through informal channels such as friends, herbalists, or family members rather than medical professionals.
- **Unsupervised Use and Lack of Medical Consultation:** The majority of individuals who had used milk thistle did so without consulting healthcare providers, highlighting a communication gap between patients and doctors regarding the use of complementary therapies.
- **Unclear Perceived Benefits:** While some users noted a slight improvement in blood glucose levels, the outcomes were inconsistent. This may be attributed to short-term or irregular use, absence of standardized dosage, or placebo effect.
- **Minimal Side Effects Reported:** Most users did not experience significant adverse effects, possibly reflecting the relatively safe profile of milk thistle or the limited duration of use.
- **High Dependence on Prescribed Medications:** Nearly all participants were undergoing conventional antidiabetic treatments, indicating that herbal remedies are largely viewed as complementary rather than alternative options.
- **Openness to Future Use if Recommended by Professionals:** Despite the limited use of milk thistle, many participants expressed a willingness to consider it if recommended by a physician or a trusted herbalist, reflecting trust in professional guidance.
- **Prevalence of Traditional Herbal Use:** When asked about other natural remedies, the majority of respondents mentioned plants such as cinnamon, artemisia, fenugreek, and olive leaves, underscoring a cultural preference for familiar, traditional botanicals.

These findings suggest that while elderly diabetic patients demonstrate limited awareness and unsupervised use of milk thistle, they remain open to its potential use under professional supervision. Their strong inclination toward well-known herbal treatments highlights the need for culturally sensitive education strategies and the integration of evidence-based traditional medicine into standard diabetes care.

2.2 Perspectives from Healthcare Professionals

To complement patient-based data, a parallel survey was conducted among healthcare professionals—five diabetes specialists and five general practitioners—to assess their knowledge and clinical stance regarding the use of *Silybum marianum* in diabetic care.

Key findings from the physician responses include:

- **Moderate Awareness but No Clinical Recommendation:** Half of the physicians were familiar with milk thistle, but none had recommended it to diabetic patients. This suggests hesitancy rooted in insufficient clinical evidence or formal guidelines.
- **Absence of Scientific Reference:** All respondents stated that they do not currently rely on clinical trials or scientific literature to support the use of milk thistle in diabetes, reflecting a disconnect between research findings and clinical practice.
- **Uncertainty About Efficacy:** Eight out of ten physicians reported being uncertain about the effectiveness of milk thistle in glycemic control, further reinforcing the need for robust scientific studies and accessible education for medical professionals.
- **Conditional Perception of Safety:** Most physicians (7 out of 10) considered milk thistle to be safe, but only **under certain conditions**—typically with medical supervision and individualized consideration of the patient's health status.
- **No Reports of Patient Use:** None of the physicians had encountered patients who used milk thistle without medical advice, possibly indicating either its limited use in the population or a lack of patient disclosure.
- **Openness to Recommendation with More Evidence:** A majority of physicians (7 out of 10) expressed willingness to consider recommending milk thistle if further scientific evidence confirmed its safety and efficacy.

These results reflect a cautious, evidence-dependent approach among healthcare providers. While current recommendation rates are non-existent, there is a clear openness to reevaluation contingent on the emergence of more substantial clinical data. Physicians also

highlighted concerns about herb-drug interactions, dosage standardization, and the risk of hypoglycemia when used alongside antidiabetic medications.

2.3 Implications

Together, these two sets of data—patient and physician perspectives—highlight a crucial gap between public interest in traditional herbal remedies and the clinical hesitance rooted in the lack of robust evidence. While patients show cultural familiarity with herbal medicine and a willingness to explore new options under professional guidance, physicians remain reserved, pending further scientific validation.

This underscores the need for:

- **Clinical trials and pharmacological research** on *Silybum marianum* specific to diabetes;
- **Educational outreach** for both patients and physicians to improve safe and evidence-based use of herbal therapies;
- **Improved communication** between patients and healthcare providers regarding complementary and alternative treatments.

By bridging this gap, there is potential to harmonize traditional knowledge with modern clinical practice in a way that is both safe and culturally sensitive

CONCLUSION

Conclusion:

In conclusion, this comprehensive exploration into the intricate relationship between Silybum marianum (milk thistle) and diabetes mellitus reveals a compelling yet complex narrative. The collective evidence synthesized within this thesis strongly suggests that silymarin, the bioactive complex derived from milk thistle seeds, possesses a range of pharmacological properties that intersect significantly with the underlying mechanisms of diabetes. Its potent antioxidant and anti-inflammatory actions, for instance, appear to combat the oxidative stress and chronic inflammation that are both contributors to and consequences of this debilitating metabolic disorder.

The reviewed literature offers intriguing insights into the potential of milk thistle to positively influence glucose homeostasis. Several studies have documented modest but noteworthy improvements in key glycemic markers, including reductions in fasting plasma glucose levels and glycated hemoglobin (HbA1c), suggesting a potential role in enhancing blood sugar control.

Furthermore, the capacity of silymarin to improve insulin sensitivity in peripheral tissues and exert a protective effect on the vulnerable pancreatic beta-cells – the insulin-producing powerhouses of the body – offers a biologically plausible rationale for its therapeutic potential in both type 1 and type 2 diabetes. By mitigating beta-cell dysfunction and preserving their functional integrity, milk thistle may contribute to sustained insulin production and secretion. However, a critical and nuanced interpretation of the existing research landscape is paramount. The heterogeneity observed across studies, encompassing variations in dosage, formulation, duration of intervention, and the characteristics of the study populations, necessitates cautious optimism.

While numerous investigations point towards beneficial effects, the presence of studies with less conclusive or even negative outcomes underscores the need for methodological rigor in future research endeavors. Large-scale, multi-center, randomized controlled trials, employing standardized milk thistle extracts and clearly defined patient stratification, are essential to definitively ascertain its clinical efficacy and establish evidence-based guidelines for its use in diabetes management.

Beyond clinical outcomes, future research should prioritize elucidating the precise molecular mechanisms through which silymarin exerts its anti-diabetic effects. Investigating its interactions with key signaling pathways involved in glucose metabolism, insulin action,

and inflammation could provide a deeper understanding of its pharmacological profile and potentially identify specific molecular targets for therapeutic intervention.

Moreover, exploring the bioavailability and pharmacokinetic properties of different silymarin formulations is crucial for optimizing its delivery and maximizing its therapeutic impact. In the broader context of diabetes care, the potential integration of milk thistle as a complementary therapy warrants careful consideration. While the findings of this thesis present a promising avenue for supportive treatment, it is imperative to emphasize that milk thistle should not be viewed as a standalone cure or a substitute for established, evidence-based medical interventions, including lifestyle modifications, pharmacological agents, and regular monitoring.

Individuals living with diabetes must engage in open and informed discussions with their healthcare providers before incorporating milk thistle or any other alternative or complementary medicine into their management regimen. Ultimately, this thesis contributes to the growing body of knowledge surrounding the potential of milk thistle in the context of diabetes.

While the preliminary evidence is encouraging, continued rigorous scientific inquiry is essential to fully unlock its therapeutic potential, define its optimal role in diabetes management, and ensure its safe and effective use for individuals striving to live well with this chronic condition.

The future of research in this area holds the promise of refining our understanding and potentially expanding the therapeutic toolkit for combating diabetes.

Recommendations

In light of the findings presented in this thesis, several key recommendations emerge to guide future research and clinical application of *Silybum marianum* in diabetes management. First, there is a critical need for well-designed, large-scale, randomized controlled trials (RCTs) employing standardized silymarin formulations to rigorously evaluate its clinical efficacy across diverse diabetic populations. These studies should ensure consistent dosing, treatment duration, and stratification of participants by diabetes type, disease duration, and comorbidities. Second, mechanistic investigations should be prioritized to elucidate the specific molecular pathways through which silymarin modulates glucose metabolism, insulin signaling, oxidative stress, and inflammatory responses. Advanced molecular and omics-based approaches may help identify novel therapeutic targets and biomarkers of response. Third, comprehensive pharmacokinetic and bioavailability studies are recommended to optimize formulation strategies that enhance silymarin absorption, stability, and tissue distribution, thereby improving its therapeutic potential. Furthermore, given the growing interest in integrative approaches, future research should also explore the safety and potential interactions between silymarin and standard antidiabetic drugs to support its safe inclusion as an adjunct therapy. Finally, public health and clinical education initiatives should promote informed decision-making among patients, encouraging dialogue between individuals with diabetes and their healthcare providers regarding the use of botanical supplements such as milk thistle. These multidisciplinary efforts are essential to establish evidence-based guidelines, refine clinical practice, and responsibly advance the use of phytotherapeutics in the management of diabetes mellitus.

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ANNEXES

Investigative Study on Milk Thistle Use in Diabetics

Questionnaire for Diabetic Patients

Q. N	Question	Response Type
1	Have you ever heard of milk thistle (Silybum marianum)?	Yes/No
2	Have you ever used milk thistle for diabetes management?	Yes/No
3	If yes, who recommended it to you?	Doctor / Herbalist / Friend or Family / Internet or Social Media / Self-researched
4	If you used milk thistle, how long have you been taking it?	Less than 1 month / 1–3 months / 3–6 months / More than 6 months
5	Have you noticed any benefits in your blood sugar levels after taking milk thistle?	Yes/No/Not Sure
6	Have you experienced any side effects from milk thistle?	Yes/No (If yes, please specify)
7	Are you currently taking any prescribed diabetes medications?	Yes/No
8	Did you consult your doctor before using milk thistle?	Yes/No
9	Would you consider continuing or starting milk thistle if recommended by a doctor or herbalist?	Yes/No/Maybe
10	What other natural or herbal remedies have you tried for diabetes?	Open-ended

Questionnaire for Doctors (Endocrinologists, General Practitioners, etc.)

Q. N	Question	Response Type
1	Are you familiar with milk thistle (Silybum marianum) and its potential health effects?	Yes/No
2	Have you ever recommended milk thistle for diabetic patients?	Yes/No
3	If no, what are the main reasons for not recommending it?	Open-ended
4	Are there any clinical trials or scientific evidence that you rely on regarding milk thistle's effects on diabetes?	Yes/No (If yes, please specify)
5	Do you think milk thistle has a significant role in blood sugar regulation?	Yes/No/Unsure
6	Do you consider milk thistle supplementation safe for diabetic patients?	Yes/No/Conditional (please explain)
7	What are the possible risks or contraindications of using milk thistle in diabetic patients?	Open-ended
8	Have any of your patients reported using milk thistle for diabetes without your recommendation?	Yes/No
9	If yes, did they report any benefits or side effects?	Open-ended
10	Would you consider recommending milk thistle if more scientific evidence supports its benefits?	Yes/No/Maybe