2.1 Introduction

Milk thistle (*Silybum marianum*) is a medicinal plant of great value as it is efficacious in a large number of human ailments. The plant is native to the Mediterranean region and grown throughout Europe, North America and Australia. The botanical name is *Silybum* Adams. (Compositae) *marianum* (L.) Gaertn. This belongs to Asteraceae family. This is a thorny, annual or biennial plant. It is called by several names like Milk thistle (English vernacular), Pavitramullina gida (Indian name), Dudhi (Hindi), Pathari (Marathi), Ratrinta (Telgu), Titaliya (Bihar), Holy thistle and Royal thistle.

In India it is distributed in N-W Himalayas, Punjab-westwards to Kashmir (Kiritikar and Basu, 1999). This is used as a traditional medicine by the European natives. In modern times its extracts are used as medicines. The main fractions are silymarin and silybin, which are used for curing several diseases. In the last 5-6 successive years cultivation has been done in the state of Rajasthan, U.P., Punjab, Haryana and M.P. The seeds of milk thistle are used in curing several diseases.

The northern Indian plains provide an ideal condition for its successful proliferation and it is expected that if the area increases then in the near future India, which presently imports the seed of milk thistle, could stop its import and very soon it could become an exporting product.

The first known report of *Silybum* was by Dioscorides, a first century Greek physician. In his ventures with the Roman army, he came across a large number of edible thistles which were varied in structure and location. Dioscorides collectively placed two plants in the genus, both
originating from the Mediterranean region. These include our species of interest, *Silybum marianum*. Dioscorides gave the name *Silybum* to a number of edible thistles. Milk refers to the white streaks along the leaf veins. In Germany, where the plant is often depicted as a religious symbol associated with the Virgin Mary, legend ascribes the white mottling to the drop of Virgin Mary’s milk. The species name “marianum” honours the symbolic association of the plant with the Virgin Mary (Foster, 1996).

Another first century Roman physician and naturalist, Pliny the Elder (23-27 A.D.) reported the use of milk thistle as a vegetable which was too troublesome to cook. However, he also mentioned the efficacy of the juice of the plant, mixed with honey, in “carrying off bile”. This was perhaps the first reference to the hepatoguard action of milk thistle (Foster, 1996).

In 1150 A.D., the woman herbalist, Hildegarde of Bingen, documented the uses of the root, whole plant and leaves of milk thistle, although her work was published as late as 1533 A.D. Culpepper (1787 A.D.) was also of the view that milk thistle is effectual “to open the obstructions of liver and spleen, thereby is good against jaundice”. Culpepper recommended boiling and eating the young plant in spring as an alterative and to improve blood circulation (Foster, 1996).

In 1929, Schutlz gave credit to an eighteenth century German physician, Rademacher, for advocating the use of milk thistle preparation in chronic liver diseases, acute hepatitis and jaundice. By the 1930s once again clinical interest in milk thistle was beginning to emerge. Intensive research into the liver protecting (hepatoguard) properties of the plant, the
responsible chemical components and mechanism of action began about 30 years ago (Foster, 1996).

Although attempts to isolate the active component of the seeds began in 1958, it was only in 1967-68 that Wagner, et al., were able to isolate a compound called silymarin. Initially, it was believed to be a single compound but was later shown to be a complex of chemicals known as flavonolignans. Improved chemical separation methods later revealed that silymarin was not a single component but a complex of chemicals known as flavonolignans. The primary components isolated and structurally characterized from silymarin include silybin (silibinin), silydianin and silychristin. Collectively, isoflavonolignans are found in concentrations of 4-6% in the ripe seeds. European milk thistle products, some of which are available on the American market, are standardized to 70-80% silymarin.

Silymarin, a flavonoid has been isolated from the seeds in a yield of 0.7% and is considered as the antihepatotoxic component of the drug. Pharmacological studies on silymarin showed that it antagonized experimentally-induced liver damage in rats. Tyramine (p-hydroxyphenethylamine), an amino acid with properties similar to adrenaline is also reported in the seeds; alcoholic extracts of the seeds have been recommended in the treatment of haemorrhoids and as a general substitute for adrenalin. Other substances in the seed include fatty oil (up to 45%), alkaloids (0.04-0.06%), a saponin, unidentified bitter substances, mucilage and organic acids (1-2%). Seeds are considered a potential source of fatty oil which may be used as food or as lubricant (Wagner and Munster, 1967).
Kingsbury 1964, observed that the plant is browsed by livestock; it is rich in calcium. It is, however, reported to be poisonous to cattle and, to a lesser extent, to sheep. The toxicity is attributed to high concentrations of nitrate, which the plant is able to accumulate particularly when grown on fertile soil. Nitrates may cause severe gastro-enteritis, but poisoning is generally due to the reduction of nitrates to the more highly toxic nitrite in the rumen of the animals.

According to Lorenz et al. (1984) in humans, the absorption half-life of the major active compound, silybin, is 17 hours, and elimination half-life is 6 hours.

Weiss (1988), confirmed the efficacy of silymarin by extensive laboratory, histological and clinical data. He also mentioned, after animal experiments, that seed extracts are safe, even in large doses, with practically no side effects, and showed no embryo toxic effect.

Ferenci et al. (1989) reported the use of *Silybum marianum* extract in the treatment of liver cirrhosis.

Morazzoni and Bombardelli (1995) reported that, in Germany, the primary causes of liver intoxication include alcohol (71 %), psychopharmaceuticals (18 %) and industrial exposure to chemicals (11 %) and silymarin is the best documented drug for treatment of liver intoxication. They also confirmed that *Silybum marianum* fruit and seed contain silymarin which is composed of an isomeric mixture of the flavonolignans, silychristin, isosilychristin, silydianin, silybin A, silybin B, isosilybin A and isosilybin B.
Flora et al. (1998) described the use of *Silybum marianum* extract in the treatment of acute and chronic toxin-alcohol and viral induced hepatitis.

According to Chopra et al. (2000), no known contraindications have been reported, and *Silybum* is even presumed safe during lactation. However, it should be avoided in cases of known allergy to plants of the Asteraceae family. Also as *Silybum* is a detoxifying herb, preliminary side effects like slight nausea, headache, skin rashes and mild diarrhoea may occur in certain individuals. These symptoms are only temporary and will soon subside.

More recently, milk thistle has been studied for applications as a chemopreventive and chemotherapeutic agent in prostate, breast, bladder, skin and ovarian cancer. (Tyagi et al., 2002; Sharma et al., 2001; Singh et al., 2002; Zi et al., 1998).

According to Kohno et al. (2002), silymarin has been proven to inhibit azoxymethane-induced colon cancer in male F344 rats.

According to Lirussi et al. (2002), in diabetes phytotherapy, flavonoids isolated from *Silybum marianum* have been demonstrated as preventive agents of cyclosporine-A induced toxicity in exocrine pancreas, protective compounds on pancreatic damage in alloxan-induced diabetes mellitus and exhibited a lowering of blood glucose level activity in type 2 diabetic patients after a long term treatment. However, until now, the hypoglycaemic activity of silymarin and silybin extracted from *Silybum marianum* has been studied only in type 2 diabetes mellitus. The results indicate a slight hypoglycaemic effect if we take into consideration the doses used and duration of treatment in type 2 diabetic patients.
Pullaiah (2002) stated that in Europe, milk thistle is used in jaundice and other biliary affectations. As a diet or in infusion it is said to be a reliable galactogogue. The herb is used for intermittent fever, dropsy and uterine troubles. A decoction of it is said to be beneficial as an external application in cancer. Leaves are sudorific and aperient.

Agarwal et al. (2003) performed a mechanism study in HT-29, a microsatellite-stable human colon cancer cell line, and showed cell cycle arrest via increased expression of cyclin dependent kinase inhibitors and the resulting apoptosis.

Sharma (2003) reported that the seeds are pungent, demulcent and antispasmodic. They are used for the treatment of jaundice and calculi of liver and gallbladder and are useful in controlling haemorrhages. Alcoholic extracts of the seed and to a lesser extent of the plant also increase peristalsis of the small intestine and galenical preparations both of the seeds and its oil are mildly purgative.

Gazak et al. (2004) confirmed the recently oxidized derivatives of silybin (the major component forming 70-80% of silymarin) and their antiradical and antioxidant activity.

According to Katiyar (2005), several studies have been conducted with silymarin against oxidative stress, inflammatory responses and benzoic peroxide-induced tumor promotion in mice. These studies have demonstrated its antioxidant, anti-inflammatory and anticarcinogenic properties. Silymarin also shows a protective role against burn-induced oxidative damage of the skin.
2.2 Review of morphological characters of milk thistle

Morazzoni and Bombardelli (1995) described the morphological characters of the plant. *S. marianum* has a stem 20-150 cm high, rarely shorter, glabrous or slightly downy, erect and branched in the upper part. The leaves are alternate, large, white-veined, glabrous with strongly spiny margins. The inflorescences are large and round capitula, solitary at the apex of the stem or its branches, surrounded by thorny bracts. The florets are hermaphrodite, tubular in shape with corolla red-purple. The fruits are hard-skinned achenes 6-8 mm long, shiny, generally brownish in colour with a white silk-like pappus at the apex. The fruits are harvested in July-August after blooming.

According to WHO monograph (1999), milk thistle (2n = 34) is an annual or biennial herb, stem 20-150 cm high, green, glabrous or slightly arachnoid-pubescent. Leaves alternate, large, glossy green, white-veined or variegated, glabrous with strongly spiny margins, basal leaves (25-50 cm long, 12-25 cm wide) cauline, pinnatifid. Inflorescence large, composed of red-purple, hermaphrodite, tubular florets gathered into a capitulum (2.5-4.0 cm in diameter), tucked in an involucre with thorny external bracts. Fruits 6-7 mm long, composed of 6-8 hard-skinned achenes with a white, silky pappus (15-20 mm in diameter) at apex.

The major active constituents are flavonolignans (1.5-3.0%), collectively known as silymarin. The major components of the silymarin complex are the four isomers silybin and isosilybin (a 1 : 1 mixture of diastereoisomers), silychristin and silydianin.
2.3 Review of cultivation and harvesting of milk thistle

According to Medd and Lovett (1979a), as cited in Groves and Kaye (1989), in milk thistle late winter and spring seedlings will behave as biennials. Germination occurs in autumn and spring. Seedling establishment is favourable after fall rains begin, particularly after a harsh summer when there is absence of grass cover, as thistle seedlings require light. Seeds remain viable for nine years or more (Sindel, 1991).

Hammouda (1993) listed the papers which discuss different water regimes and nitrogen fertilization levels in order to obtain the highest silymarin content in the plant for its commercial production for pharmaceutical use. In Egypt the plant was cultivated and subjected to different agricultural conditions including the effect of water regime 70%, 60% and 45% per field capacity and nitrogen fertilization levels 0, 50, 100, and 150 kg/feddan. The fruits grown under the seven treatments were collected separately and subjected to investigation in comparison with the two wild growing types of *S. marianum*. Cultivated *Silybum marianum* yielded increased silymarin content when compared to wild *Silybum*. Higher results were obtained with 60% water regime per field capacity without fertilization. When soil was treated with different nitrogen fertilization levels, the 100 and 150 kg nitrogen per feddan gave the highest silymarin contents in the fruits (1.46%, 1.42% respectively).

According to Omer *et al.* (1993), nitrogen and potassium fertilizers and plant spacing affected seed yield and seed content of the active, antihapatotoxic flavonolignans silybin, silidianin and silicristin of *Silybum marianum*. A narrow row spacing (25 cm) produced higher yields of seeds, but reduced oil and flavonolignan content as compared
with a wide row spacing (50 cm). Potassium fertilizer at 115 kg K₂O/ha and nitrogen fertilizer at 140 kg N/ha increased seed yield, oil yield and percent oil and flavonolignan content of seeds as compared with lower rates of fertilizer. Cultivation of Silybum marianum at spacing of 50 cm between rows and with fertilizer at 55 kg K₂O/ha yielded the highest percentage of silybin in Silybum marianum seeds. Nitrogen had no effect on the percentage of silybin seeds.

According to Thomas (2000), in his book Medicinal Plants, milk thistle is planted at a spacing of 40 cm. and he also stated that the major disease in the plant is powdery mildew caused by Erysiphe cichoracearum, and the major insect which destroys the crop, is Nezara viridula.

According to Farooqi and Sreeramu (2001), the crop is raised by using the seeds obtained from the previous season’s crop. It is sown during October and November in the plains and in March to April in hilly areas. The seeds are either sown by broadcasting them in rows spaced at 60 cm or they can also be sown at 60×60 cm. spacing using a seed drill.

They also recommend the application of manure and fertilizers: FYM at the rate of about 8-10 t/ha, 120 kg N, 100 kg P₂O₅ and 75 kg K₂O/ ha. They stress that it is a sufficiently hardy crop and does not require very frequent irrigation, except during the early stages of growth and that Silybum is practically free from most pests and diseases.

Farooqi and Sreeramu, also stated that if harvested at the right time, on an average, about 1 t/ha of seeds may be obtained.
Omidbaigi and Nobakht 2001, stated that milk thistle is an annual over wintering plant belonging to Asteraceae family that reaches a height of 200-250 cm. The capitula are 5-8 cm in diameter and ovate. The flowers are purple in colour. The sunny, stony slopes of the Mediterranean region are the growing locations of warmth-loving milk thistle. It is common in countries of the Mediterranean region. The ripe fruit of milk thistle contains flavonoids. The results of Omidbaigi and Nobakht stated that the milk thistle productivity is strongly affected by nitrogen fertilizer.

The nitrogen dosage were used in the form of urea (50, 120 and 200 kg/hectare) and with this 250 kg/hectare P$_2$O$_5$ was applied. The distance was maintained 50 cm between the rows and 30 cm between each successive plant. Results of these experiments were shown in the form of significant increase in the plant height, capitula number per plant, capitula diameter in full flowering stage, weight of 1000 seeds, seed yield, silymarin and silybin content. Plant height significantly increase upto 38% in comparison to control treatment, the largest capitula diameter 4.76 cm and the number of seeds per capitula (123.4) were obtained in the plot of 200 kg/hectare nitrogen. In this plot seed yield was also increased upto 85.1% as compared with the control treatment but nitrogen fertilization had negative significant effect on percentage of silymarin and silybin. The highest amount of silymarin (9.92%) and silybin (33.58%) was accumulated in the seeds of control treatment plants.

According to Kuepper et al. (2003), foliar feeding has been used as a means of supplying supplemental doses of minor and major nutrients, plant hormones, stimulants, and other beneficial substances. Observed effects of foliar fertilization have included yield increases, resistance to diseases and insect pests, improved drought tolerance, and enhanced crop
quality. Plant response is dependent on species, fertilizer form, concentration, and frequency of application, as well as the stage of plant growth.

Wojcik (2004) reported that foliar fertilization of plants is a valuable complement to the application of nutrients to the soil. Foliar fertilization is most effective when soil nutrient availability is low, topsoil dry, and root activity during the reproductive stage is decreased. Foliar fertilization is also successful in increasing content of fruit Ca$^{2+}$ and cereal grain protein. It is proposed that this treatment should be recommended in the integrated plant production because it is environment friendly and increases productivity and yield quality. Wojcik also describes the factors which influencing absorption of mineral nutrients.

In a study by Ram et al. (2005), 10 exotic and 5 indigenous collections were collected from different places and grown in Jammu region (India). The seeds were directly sown in lines 50 cm apart in well prepared field beds (9×5 m), in the month of October in triple rows in plots of 6 m length with inter-and-intra row spacing of 50 and 30 cm. Weeding and thinning were done after 30 days of plantation. Fifteen accessions (10 exotic and 5 indigenous) of S. marianum were assessed for plant height, stem diameter, plant canopy, number of capsules/plant, capsule diameter, seed yield/plant and silymarin content to evaluate genetic variability. Out of all the collections the highest silymarin content, 3.56 %, was obtained in the local variety RLSM-14.

According to Valtcho et al. (2006), a limiting factor in blessed thistle production is weed interference. Field experiments were conducted near
Plovdiv, Bulgaria, to study the effect of selected herbicides on weed control, crop productivity, and crop quality. Seed yields of blessed thistle were increased with metribuzin alone at 0.5 kg ai/ha, pendimethalin alone 1.32 kg ai/ha, pendimethalin at 1.32 kg ai/ha plus metribuzin at 0.5 kg ai/ha, trifluralin at 0.84 kg ai/ha plus linuron at 1.0 kg ai/ha, and in the hand-weeded control compared to the nonweeded control (nontreated check). The results stated that weed control increased the content of silymarin and decreased the amount of seed oil. Overall, seeds contained 0.26-0.36% taxifolin, 0.69-0.99% silydianin plus silycristin, 1.31-1.78% silybin, and 0.27-0.39% isosilybin.

As cited in Haj Sayed Hadi et al. (2008), treatments were done in 2 production systems (conventional and low input system), and the results showed that there was a significant difference between production systems. The highest height (125.8 cm) and number of capitols per plant (10.4) were obtained in conventional system. While other traits including capitol diameter (7.028 cm), number of seed per capitol (125), 1000 seed weight (25.006 g), seed yield (1888.072kg/ha), silymarin percentage (7.711%) and silymarin yield (150.44 lit/ha) were recorded in the low input system.

Geneva et al. (2008) described the effects of foliar or soil fertilization and MD 48/II (3-methylphenylamide 5-tert-butylypyrazine-2-carboxylic acid) on the vegetative and reproductive growth. Some physiological parameters, seed yield and silymarin content of field grown milk thistle plants were studied. The highest silymarin level (5.876% of dry matter) was assayed in the seeds of control plants.
2.4 Quantitative analysis of silymarin in milk thistle

Lorenz et al. (1984) analysed the flavonoids of silymarin by thin layer chromatography (TLC).

Martinelli et al. (1991) analysed the flavonoids of silymarin by high performance liquid chromatography (HPLC).

Mascher et al. (1993) analysed the flavonoids of silymarin by high performance liquid chromatography (HPLC).

According to Wichtl (1994), the dried seeds contain 1-4% of silymarin flavonoids. Silymarin is a mixture of three flavonolignans, including silybin (silibinin), silidianin, and silichrysin. Other flavonolignans identified in S. marianum include dehydrosilybin, deoxysilycristin, deoxysilydianin, silandrin, silybinome, silyhermin, and neosilyhermin. In addition, milk thistle contains apigenin, taxifolin, silybonol, myristic, oleic, palmitic and stearic acids.

Rickling et al. (1995) analysed the flavonoids of silymarin by high performance liquid chromatography (HPLC).