Milk thistle extract (*Silybum marianum*) extract

Milk thistle (*Silybum marianum*) is a medicinal plant from the Asteraceae family. Silymarin is the major constituent of milk thistle extract and is a mixture of some flavonolignans such as silybin, which is the most active component of silymarin. It is most commonly known for its hepatoprotective effect. Also, studies have shown other therapeutic effects such as anticancer, anti-Alzheimer, anti-Parkinson, and anti-diabetic, so its safety is very important. It has no major toxicity in animals. Silymarin has low-drug interactions, and it does not have major effects on cytochromes P-450. Some studies demonstrated that the use of silymarin must be with caution when co-administered with narrow therapeutic window drugs[1].

Silymarin represents one of the best examples how a herbal preparation can be developed from the traditional herbal medicine[2].The clinical use of silymarin in the treatment of NAFLD is experimentally supported by its anti-inflammatory, antioxidant, antifibrotic, and proregenerative effects, as well as by its metabolic actions on insulin resistance and hyperlipidaemia[3-5].

In another study[6], the effects of *Silybum marianum* oil (SMO) on hepatic steatosis and oxidative stress were investigated during the development of nonalcoholic fatty liver disease (NAFLD) in high fat diet (HFD)-fed mice. The results showed that body weight, fat mass, and serum biochemical parameters such as triglyceride, free fatty acid, glucose and insulin were reduced by SMO treatment. Meanwhile, SMO decreased the histological injury of liver and the levels of hepatic triglyceride, cholesterol and free fatty acid in HFD-fed mice. SMO administration elevated the activities of superoxide dismutase (SOD) and catalase (CAT) and reduced the level of malondialdehyde (MDA) in the liver. The results indicated that SMO could play a certain protective role against HFD-induced NAFLD, and the protective effects might be associated with attenuating lipid accumulation, oxidative stress and inflammation, improving lipid metabolism.

Silymarin has received a tremendous amount of attention over the last decade as a herbal remedy for liver treatment. In many cases, the antioxidant properties of silymarin are considered to be responsible for its protective actions[7]. Direct scavenging free radicals and chelating free Fe and Cu are mainly effective in the gut. Preventing free radical formation by inhibiting specific ROS-producing enzymes, or improving an integrity of mitochondria in stress conditions, are of great importance. Maintaining an optimal redox balance in the cell by activating a range of antioxidant enzymes and non-enzymatic antioxidants, mainly via Nrf2 activation is probably the main driving force of antioxidant (AO) action of SM. Decreasing inflammatory responses by inhibiting NF-κB pathways is an emerging mechanism of SM protective effects in liver toxicity and various liver diseases. Activating vitagenes, responsible for synthesis of protective molecules, including heat shock proteins (HSPs), thioredoxin and sirtuins and providing additional protection in stress conditions deserves more attention. Affecting the microenvironment of the gut, including SM-bacteria interactions, awaits future investigations. In animal nutrition and disease prevention strategy, SM alone, or in combination with other hepato-active compounds (carnitine, betaine, vitamin B12, etc.), might have similar hepatoprotective effects as described in human nutrition.

Though silymarin does not have antiviral properties against hepatitis virus, it promotes protein synthesis, helps in regenerating liver tissue, controls inflammation, enhances glucuronidation and protects against glutathione depletion. Silymarin may prove to be a useful drug for hepatoprotection in hepatobiliary diseases and in hepatotoxicity due to drugs. The non-traditional use of silymarin may make a breakthrough as a new approach to protect other organs in addition to liver. As it is having a good safety profile, better patient tolerability and an

effective drug at an affordable price, in near future new derivatives or new combinations of this drug may prove to be useful[8].

Silybum marianum (SM, 1.5 mg/kg live weight as silybin) was tested for its health effects on 8 dogs with hepatopathy[9]. The group of dogs allotted to SM treatment suffered from liver diseases as also confirmed by higher plasma ALT activity at T0. The significant reduction of this enzyme activity at T60 is consistent with the well-known liver protectant activity, which was observed also by previous studies.

In an extensive clinical review on Silymarin's properties Wellington and Jarvis (2001)[10] explained which are the different mechanisms involved in its biological activity. They indicated the increasing in the synthesis of ribosomal RNA (rRNA) species through stimulation of polymerase I and rRNA transcription (thereby increasing the synthetic rate of structural and functional proteins), the blockage of the uptake of toxins such as α-amanitin from *Amanita phalloides* (the deathcap mushroom), and the protection of the cell membrane from osmotic stress and radical-induced damage.

Artichoke (*Cynara scolymus*) extract

Cynara scolymus L. (Artichoke) has been used for the treatment of metabolic disorders. The hepatoprotective effect of *Cynara scolymus* leaves extract against a high fat diet (HFD) induced rats was studied[11], focusing on the most abundant phenolic compounds rich *Cynara scolymus* leaves extract and its antihypercholesterolemic and antioxidative effects in vivo. The hypercaloric high fat diet (HFD) was treated with 200 mg/kg and 400 mg/kg of ethanol extract (EEA) from leaves of Cynara and atorvastatin (ATOR) (10 mg/kg/day) during an 8-week period. Lipid profile was measured and oxidative stress systematic in hepatic tissue was determined. Our data revealed that HFD-induced hepatic dysfunction manifested by significant abnormal levels of AST, ALT, ALP, LDH, and OCT was accompanied by increasing levels of oxidative stress biomarker (ROS, MDA, and AOPP) while decreasing in antioxidant status. Coadministration of EEA significantly reduced serum lipid profile and hepatic disorders which was confirmed to be histological by reducing the fatty liver deposition in hepatic lobule. These findings suggest that Cynara leaves exert antiobesity and antioxidant liver effects in HFD-induced obese rats.[11]

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide and is potentially treatable, though there are few therapeutic agents available. Artichoke leaf extract (ALE) has shown potential as a hepatoprotective agent. During a randomized double-blind placebo-controlled parallel-group trial[12], ALE was examined for therapeutic utility in patients with established NAFLD. 100 subjects with ultrasound-diagnosed NAFLD were randomized to either ALE 600 mg daily or placebo for a 2-month period. NAFLD response was assessed by liver ultrasound and serological markers including the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio and AST to platelet ratio index (APRI) score. ALE supplementation reduced total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and triglyceride concentrations ($p = .01$). This study has shown beneficial effects of ALE supplementation on both ultrasound liver parameters and liver serum parameters (ALT, AST, APRI ratio, and total bilirubin) in patients with NAFLD.

Oxidative stress and inflammation are well-documented pathological factors in alcoholic liver disease (ALD). The ethanolic extract from artichoke has also been evaluated in preventing against acute alcohol-induced liver injury in mice. Male mice were treated with an ethanolic extract of artichoke (0.4, 0.8, and 1.6 g/kg body weight) once daily. [13]. Up to 40% alcohol (12 mL/kg body weight) was administered orally 1 h after artichoke treatment. All mice were fed

for 10 consecutive days. Results showed that artichoke extract significantly prevented elevated levels of aspartate aminotransferase, alanine aminotransferase, triglyceride, total cholesterol, and malondialdehyde. Meanwhile, the decreased levels of superoxide dismutase and glutathione were elevated by artichoke administration. Histopathological examination showed that artichoke attenuated degeneration, inflammatory infiltration and necrosis of hepatocytes. Immunohistochemical analysis revealed that expression levels of toll-like receptor (TLR) 4 and nuclear factor-kappa B (NF-κB) in liver tissues were significantly suppressed by artichoke treatment. Results obtained demonstrated that artichoke extract exhibited significant preventive protective effect against acute alcohol-induced liver injury. This finding is mainly attributed to its ability to attenuate oxidative stress and suppress the TLR4/NF-κB inflammatory pathway

Hepatocurative effects of *C. scolymus* leaf extract on carbon tetrachloride (CCl4)-induced oxidative stress and hepatic injury in rats were investigated by serum hepatic enzyme levels, oxidative stress indicator (malondialdehyde-MDA), endogenous antioxidants, DNA fragmentation, p53, caspase 3 and histopathology[14]. Animals were divided into six groups: control, olive oil, CCl4, C. scolymus leaf extract, recovery and curative. CCl4 was administered at a dose of 0.2 mL/kg twice daily on CCl4, recovery and curative groups. *Cynara scolymus* extract was given orally for 2 weeks at a dose of 1.5 g/kg after CCl4 application on the curative group. Significant decrease of serum alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) levels were determined in the curative group. MDA levels were significantly lower in the curative group. Results indicated that *C. scolymus* leaf extract has hepatocurative effects of on CCl4-induced oxidative stress and hepatic injury by reducing lipid peroxidation, providing affected antioxidant systems towards the normal range. It also had positive effects on the pathway of the regulatory mechanism allowing repair of DNA damage on CCl4-induced hepatotoxicity.

In a study[15] looking to evaluate the protective effect of the ethanol leaves extract of *C. scolymus* in alloxan induced stress oxidant, hepatic-kidney dysfunction and histological changes in liver, kidney and pancreas of different experimental groups of rats, the extract was found to have anti-hyperglycemic properties, at least partly mediated by antioxidant and hypolipidemic effects.

L-Methionine

Methionine is an essential AA for animals. The sulfur-containing side chain makes Met a major AA not only for protein synthesis but also for other various biological functions. Previous research investigated the functional role of Met, including methyl donation[16], antioxidative effects[17-18], and a precursor of bioactive compounds such as glutathione (GSH) and taurine[16].The various functional roles of Met are important for the development and health status of animals[19-21].

Prolonged administration of high-cholesterol diet not only disturbs the structure of cell membranes, which is expressed by decreased activity of enzymes in the liver and the migration of those enzymes to plasma but as well leads to steatosis of the liver, which has been confirmed by histological examinations. The influence of methionine, selenomethionine, and vitamin E on liver metabolic pathways and steatosis were studied and the results showed a beneficial influence of methionine and vitamin E supplementation on liver steatosis development[22].

Moreover, the relative protective abilities of silymarin and l-methionine pre-treatment in acetaminophen overdose injuries of the liver, kidney and cerebral cortex were compared by

assessing behaviours, antioxidant status, tissue histological changes and biochemical parameters of hepatic/renal function[23]. Plasma was assayed for biochemical markers of liver/kidney function; while sections of the liver, kidney and cerebral cortex were either homogenised for assay of antioxidant status or processed for histology. Acetaminophen overdose resulted in locomotor retardation, excessive self-grooming, working-memory impairment, anxiety, derangement of liver/kidney biochemistry, antioxidant imbalance, and histological changes in the liver, kidney and cerebral cortex. Administration of silymarin or increasing doses of l-methionine counteracted the behavioural changes, reversed biochemical indices of liver/kidney injury, and improved antioxidant activity. Silymarin and l-methionine also conferred variable degrees of tissue protection, on histology. Either silymarin or l-methionine can protect vulnerable tissues from acetaminophen overdose injury; however, each offers variable protection to different tissues.

Vitamin B6

For many years the sole function of vitamin B6 was considered to be that of an enzymatic cofactor. However, it has also become clear that it is also a potent antioxidant that effectively quenches reactive oxygen species and is thus of high importance for cellular well-being.

In a recent study[24], the detoxifying effect of pyridoxine against acetaminophen (APAP)-induced hepatotoxicity was investigated. HepG2 cells were co-treated with APAP and pyridoxine to compare with betaine or methionine for 24 h. LDH, ALT and AST activities were measured to determine direct cells damage in vitro and in vivo. Lipid peroxidation, antioxidant enzymes activity, and glutathione level were measured. Significant increases in activities of GST and GPx were observed after co-treatment with APAP and pyridoxine. The protective effects of pyridoxine against APAP-induced hepatoxicity were closely associated with suppression of APAP-induced oxidative stress and apoptotic cell death in HepG2 cells. These data indicated that the protective action of pyridoxine against hepatic cell injuries was involved in the direct antioxidant activity which provides a pivotal mechanism for its potential hepatoprotective action.
Vitamin B6 may directly or indirectly play a role in oxidative stress and the antioxidant defense system. Mice with deficient vitamin B6 intakes had an aggravate effect under homocysteine-induced oxidative stress[25]. In another study, the efficacy of vitamin B6 against chromium (Cr)-induced oxidative stress was examined[26]. Both pre- and simultaneous treatments countered Cr-induced oxidative stress; pre-treatment was more effective than concurrent administration. The results demonstrate the antioxidant potential of vitamin B6.

Vitamin B12

Altered vitamin B12 levels have been correlated with hepatotoxicity. Elevation in the levels of aminotransferases, SALP, total bilirubin and HP was observed in N'-nitrosodimethylamine treated rats, which was concomitant with remarkable depletion in liver glycogen and B12 reserves ($p < 0.05$)[27]. Liver biopsies also demonstrated disrupted lobular architecture, collagen amassing and intense fibrosis by NDMA treatment. Immunohistochemical staining showed the presence of activated stellate cells that was dramatically increased up to day 21 in fibrotic rats. Following vitamin B12 treatment, liver function biomarkers, glycogen contents and hepatic vitamin B12 reserves were restored in fibrotic rats, significantly. Vitamin B12 administration also facilitated restoration of normal liver architecture.

The present study elucidated the protective role of vitamin B12 with folic acid against arsenic-induced hepatotoxicity in female rats[28]. Ingestion of sodium-arsenite- contaminated water [0.4 ppm/100 g body weight (b.w.)/day] in combination with vitamin B(12) plus folic acid (0.07 and 4.0 μg, respectively/100 g b.w./day) for 24 days to Wistar rats offered a significant protection against alone arsenic-induced distorted liver function, damaged histoarchitecture, elevated oxidative stress, and DNA fragmentation of hepatic tissues. Vitamin supplementation restrained the increase of TBARS and CDs by restoring catalase, SOD, and NPSH levels. Restricted generation of free radicals may be correlated to the protection of DNA stability and hepatic morphology. This study explains the decisive role of vitamin B12 with folic acid to ameliorate arsenic-mediated liver injuries.
Deficiencies in vitamin B12 are common in cats with chronic gastrointestinal disease and require treatment with parental cobalamin[29].

A-lipoic acid

a-Lipoic acid is readily absorbed from the diet. It is rapidly converted to DHLA in many tissues. One or both of the components of the redox couple effectively quench a number of free radicals in both lipid and aqueous domains. Both DHLA and a-lipoic acid have metal-chelating activity. DHLA acts synergistically with other antioxidants, indicating that it is capable of regenerating other antioxidants from their radical or inactive forms[30].
Recent evidence indicates that alpha-lipoic acid (α-LA) has a variety of liver-protective effects through the suppression of inflammatory mediators including tumor necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS). The beneficial effects of α-LA were studied in a rat model of acute liver injury and to clarify the mechanisms of α-LA action[31]. A single injection of α-LA improved the survival rate by more than 80%. α-LA prevented serum transaminase increases, histopathologic changes, and apoptosis in the liver. α-LA decreased the expression of iNOS mRNA and its antisense transcript, leading to the reduction of iNOS protein expression and resulting in the inhibition of nitric oxide production. An electrophoretic mobility shift assay revealed that α-LA reduced the activation of nuclear factor-kappa B induced by GalN and LPS. α-LA inhibited the induction of inflammatory mediators, such as TNF-α and iNOS, in part through the inhibition of nuclear factor-kappa B activation and enhanced the induction of IL-10.
In another study[32], the viability of alpha-lipoic acid (α-LA) in ensuring against carbon tetrachloride (CCl4)-actuated liver fibrosis and the mechanism(s) involved in this defensive impact were considered in rats. All negative impacts were all restrained by α-LA, which could be a compelling means of forestalling collagen deposition and hepatic oxidative stress as well as downregulating the expression of hepatic proinflammatory cytokines, iNOS, and NF-κB and upregulating MMP-13 expression.
As a food additive, α-lipoic acid resulted in increased ratios of reduced white blood cells to oxidized forms in dogs[33]. Administration to cats prolongs elimination of α-lipoic acid compared to that of other species; therefore, administration rates should be adjusted accordingly[34].

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