Biochemical bases of the pharmacological action of the flavonoid silymarin and of its structural isomer silibinin

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The flavonoid silymarin and one of its structural components, silibinin, have been well characterized as hepato-protective substances. However, little is known about the biochemical mechanisms of action of these substances. This review deals with recent investigations to elucidate the molecular action of the flavonoid. Three levels of action have been proposed for silymarin in experimental animals: a) as an antioxidant, by scavenging prooxidant free radicals and by increasing the intracellular concentration of the tripeptide glutathione; b) regulatory action of the cellular membrane permeability and increase of its stability against xenobiotic injury; c) at the nuclear expression, by increasing the synthesis of ribosomal RNA by stimulating DNA polymerase I and by exerting a steroid-like regulatory action on DNA transcription. The specific hepatoprotective action of silibinin against the toxicity of ethanol, phenylhydrazine and acetaminophen is also discussed. It is suggested that the biochemical effects observed for the flavonoid in experimental models may settle the basis for understanding the pharmacological action of silymarin and silibinin.

Key words: *free radical scavenger action, hepatoprotective flavonoids, natural antioxidants, silibinin, silymarin.*

INTRODUCTION

Flavonoids are plant products belonging to the family of the benzo-gamma-pyrones and are mostly abundant in the photosynthetic cells of higher plants (Havsteen, 1983). More than 500 different types of flavonoids are now known, being ubiquitous both in the plant and in the animal kingdoms. Flavonoids, when incorporated into the alimentary chain may also be present in insects, molluscs, reptiles, and even mammals (Middleton, 1984). For centuries, a number of different therapeutic and curative properties have been ascribed to flavonoids and many of them have been incorporated to the popular folk medicine. Flavonoids such as quercetin (Beretz *et al*, 1982), taxifolin (Vladutiu *et al*, 1986) and silymarin (Vogel, 1968) have been used as pharmacological principles, either as such, or mixed in several chemically complex preparations. Of these flavonoids, the flavonolignane silymarin, introduced as a "hepatoprotective» agent a few years ago (Koch and Tschemy, 1983), is the best known because of its well defined therapeutic and prophylactic properties.

Silymarin which is extracted from the seeds and fruits of the milk thistle *Silybum marianum*, Gaertner (Compositae), is a mixture of three structural isomers, silibinin, silidianin, and silichristin (Koch *et al*, 1980). The structures of the isomers of silymarin were elucidated by Wagner *et al* (1965) and by Pelter and Hansel (1968). Figure 1 shows the chemical structure of the three isomers composing silymarin. A feature that distinguishes silymarin from other flavonoids is

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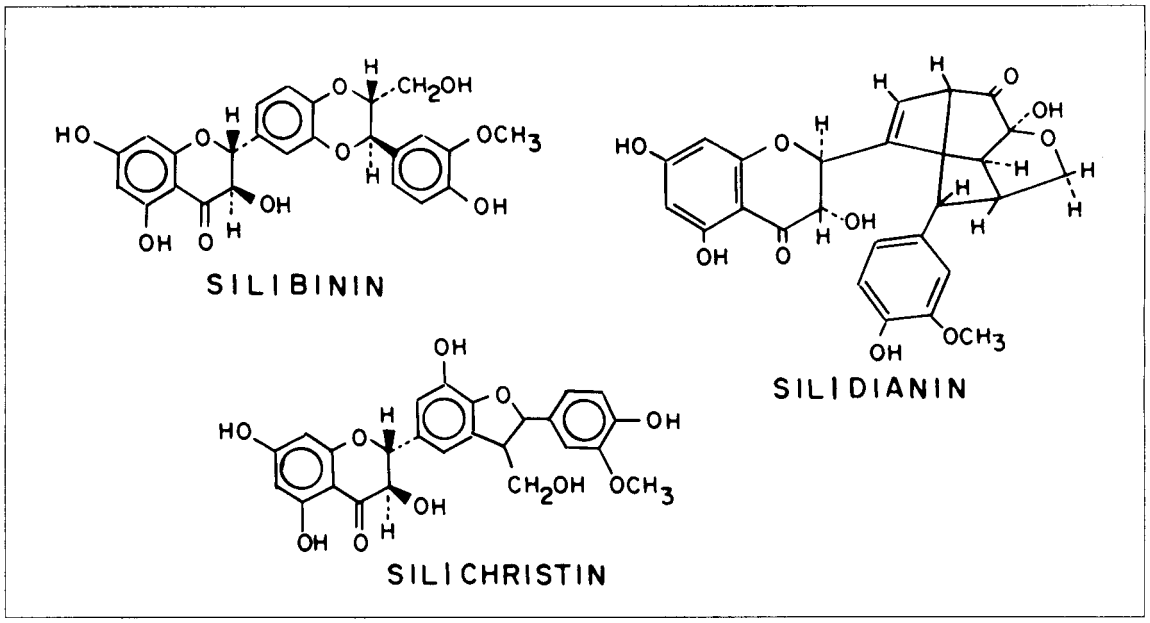

Fig 1. Chemical structure of the isomers forming silymarin.

that its isomers are always substituted by coniferylic alcohol (Wagner *et al*, 1974). Of the isomers composing silymarin, silibinin (formerly named silybin) shows the higher pharmacological potency when compared to those of silidianin and silichristin (Lecompte, 1975). Medically, silymarin and silibinin have been defined as cytoprotective substances (Stockinger *et al*, 1976) and specifically as hepatoprotective principles (Vogel *et al*, 1977). The flavonoid is used in the clinical treatment of several hepatopathies where degenerative necrosis and functional impairment are involved (Lecompte, 1975a). Silymarin is also an effective antidote against intoxication by the mushroom *Amanita phalloides* (Choppin and Desplaces, 1979). Silymarin also shows hepatoprotective effects against the intoxication with phalloidin (Vogel, 1981), galactosamine (Barberino *et al*, 1977), thioacetamide (Schriewer *et al*, 1973), halothane (Janiak, 1974), and carbon tetrachloride (Dubin *et al*, 1976).

Although silymarin has been incorporated to the pharmacopeia of many countries as Legalon® or Hepatron®, being prescribed for the treatment of a great variety of diseases, little is known about the biochemical basis of its mechanism of action. Formerly, silymarin was described as a "membrane stabilizing substance" by Schriewer and Rauen (1971), referring to its possible antioxidant capacity.

The present review deals with the efforts of different researchers, including ourselves, towards the elucidation of the protective action of silymarin and silibinin at the cellular level. Results discussed here were obtained from different *in vivo* and *in vitro* experimental models, such as whole animals, perfused organs, cells tissue homogenates and isolated nuclei. Although no definitive conclusions may be drawn about the molecular action of silymarin (or silibinin), a general hypothesis for the mechanisms of action of the flavonoid may be proposed.

I. ANTIOXIDANT PROPERTIES OF SILIBININ

Flavonoids are generally recognized as good antioxidant compounds (Fraga *et al*, 1987). The presence of hydroxyl groups in different positions of their benzene rings makes feasible hydrogen abstraction for neutralizing free radicals (Nieto *et al*, 1993). Silibinin, when assayed as the hydro-soluble silibinin-2,3'-dihydrogensuccinate sodium salt, shows a potent inhibitory effect against the oxidation of a linoleic acid:water emulsion when catalyzed by Fe^{2+} (Valenzuela and Guerra, 1986). The observed antioxidant

action is even more efficient than that of two well known synthetic antioxidants, butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) (Valenzuela *et al*, 1986). However, when the antioxidant effect of the flavonoid is assayed against complex oxidizable systems, such as the NADPH-Fe^{2+}-ADP or the ter-butyl hydroperoxide (TBH)-induced hepatic microsomal peroxidation, different results are obtained. Silibinin exhibits a good concentration-dependent inhibitory effect of the microsomal peroxidation induced by NADPH-Fe^{2+}-ADP (Valenzuela and Guerra, 1986), a well known hydroxyl free radical forming-system (Svingen *et al*, 1979), when the peroxidation is measured either as accumulation of thiobarbituric acid reactive substances (TBARS) or as spontaneous chemiluminescence (QL) (Valenzuela and Guerra, 1986). However, silibinin is unable to inhibit the peroxidation induced by TBH to the same microsomal preparation (Valenzuela and Guerra, 1986). Therefore, it seems that silibinin may only scavenge low molecular weight free radicals, as the hydroxyl free radical, being unable to neutralize more bulky free radicals as the terbutoxy free radical (Valenzuela and Guerra, 1986).

II. PROTECTIVE EFFECT OF SILIBININ IN BIOLOGICAL MODELS WHERE OXIDATIVE STRESS IS INDUCED

Oxidative stress is described as the structural and/or functional damage produced on a tissue by the uncontrolled formation of prooxidant oxygen free radicals (Sies, 1986). Generally, oxidative stress is developed when the prooxidant action of an inducer (enzyme, xenobiotic or metal) exceeds the antioxidant capacity of the cellular defense system, surpassing its homeostatic capability and eventually leading to their death (Weiss *et al*, 1982). Many xenobiotics have been characterized as oxidative stress-inducers, being the effects of carbon tetrachloride (CCl_4) (Comporti, 1985), TBH (Minotti, 1989), ethanol (Valenzuela *et al*, 1980), acetaminophen (Mason and Fischer, 1986) and phenylhydrazine (Valenzuela *et al*, 1977) among the best characterized. Erythrocytes obtained from rats treated with silymarin show a high resistance against the hemolytic effect of phenylhydrazine (Valenzuela *et al*, 1987) and against the lytic effect of osmotic shock (Valenzuela *et al*, 1985a). This latter effect suggests that silymarin might act by increasing the stability of the erythrocyte membrane. However, the molecular interaction of the flavonoid with erythrocyte membranes obtained from rats treated with silymarin has not yet been assayed. Interestingly the stimulatory action of the flavonoid on the permeability of rat bone-marrow cells to labelled substrates *in vitro* has been demonstrated. The permeability of isolated rat bone marrow cells to ^{3}H-uridine is greatly enhanced when animals are previously treated with silymarin (Garrido *et al*, 1988). This stimulatory effect is observed both in the acid-soluble fraction (cytoplasmatic ^{3}H-uridine) and in the acid-insoluble fraction (RNA), suggesting that the flavonoid enhances the transport of the radioactive precursor to the cytoplasm and its incorporation into the nucleic acids.

Liver perfusion can be a valuable experimental tool to assay both the effect of oxidative stress-inducers and the protective action of free radical-scavenger substances (Valenzuela and Guerra, 1985). Perfusion of isolated rat livers with a solution containing phenylhydrazine results in an increase in the oxygen consumption of the organ and in the release of TBARS to the caval perfusate (Valenzuela and Guerra, 1985). Such stress is accompanied by a substantial reduction of the glutathione (reduced form, GSH) content of the liver (Valenzuela *et al*, 1985b). GSH has been identified as an important protective biomolecule against chemically-induced oxidative stress (Videla and Valenzuela, 1982).

When rats are previously treated *in vivo* with silibinin (50 mg/kg iv), a significant reduction in the phenylhydrazine-stimulated oxygen consumption of the liver is observed (Valenzuela and Guerra, 1985). In addition, as a result of the flavonoid administration, the release of TBARS to the perfusing solution is substantially reduced, where as the GSH content of the tissue remains unchanged (Valenzuela and Guerra, 1985). The antioxidative effect of silibinin has also been observed in rats acutely intoxicated either with ethanol (Videla *et al*, 1982) or with

acetaminophen (Campos *et al*, 1989). Both xenobiotics have been characterized as hepatic lipid peroxidation-inducers and as drastic liver GSH-depletors (Videla *et al*, 1980; Wendel *et al*, 1979). Treatment of rats with either silymarin (Valenzuela *et al*, 1985b) or silibinin (Campos *et al*, 1988) protects animals against the hepatic oxidative stress induced by an acute intoxication with ethanol (5 g/kg b w) or with acetaminophen (50 mg/kg ip). In addition, silibinin treatment attenuates the substantial increases in the plasmatic levels of the enzymes glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase (Garrido *et al*, 1989), as well as the activity of gamma-glutamyl transpeptidase (Muriel *et al*, 1994), observed after acetaminophen intoxication (Mitchell *et al*, 1973). The levels of these enzymes are generally considered to be good markers of the hepatic function (Black, 1980).

It has been reported that acetaminophen, when administered in high concentrations, exhibits antioxidant properties upon both *in vivo* and *in vitro* experimental models (DuBois *et al*, 1983). Isolated rat hepatocytes incubated with acetaminophen (over 5 mM) show a drastic reduction in their GSH content, lipid peroxidation being not observed (TBARS values significantly lower than controls) (Garrido *et al*, 1991). When acetaminophen-treated hepatocytes are also incubated with silibinin, a potentiation of the antilipoperoxidative effect of acetaminophen is observed, as well as a protective effect on the GSH depletion induced by the drug (Garrido *et al*, 1991). This paradoxical behaviour is explained as follows: acetaminophen-induced lipid peroxidation has been related to the prooxidative action of the electrophilic reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) (Corcoran *et al*, 1980), which is formed when acetaminophen undergoes oxidative metabolism at the microsomal cytochrome P-450 system (Potter *et al*, 1987). NAPQI may react with cellular macromolecules, eventually leading to cell death (Tsokos-Kuhn *et al*, 1988). Silibinin, as several flavonoids (Beyeler *et al*, 1988), is a competitive and reversible inhibitor of the microsomal cytochrome P-450 system (Bindoli *et al*, 1977). Thereby, in the presence of silibinin, the microsomal transformation of acetaminophen to NAPQI should be inhibited; this inhibition may in turn induce an increase in the intracellular concentration of the non-metabolized acetaminophen molecules, avoiding the GSH consumption by the drug metabolite (NAPQI), which reacts with GSH forming conjugation adducts (Garrido *et al*, 1991). This effect may explain the protective action observed for the flavonoid on the hepatocyte GSH depletion when cells are incubated with acetaminophen. Moreover, nonmetabolized acetaminophen molecules may be acting themselves as free radical scavengers, enhancing the antioxidative action of silibinin.

Carbon tetrachloride-induced liver cirrhosis has been used as another biological model to assess the hepatoprotective action of silibinin in rats (Mourelle *et al*, 1989). It is known that chronic treatment of rats with CCl_4 produces liver fibrosis and a set of biochemical and histological changes that closely resemble most aspects of human portal cirrhosis (Guengerich, 1990). Using this toxicological model, Muriel and Mourelle (1990) demonstrated that silibinin may prevent the changes in the phospholipid composition of the hepatic membranes induced by CCl_4 intoxication, preserving the functional and the structural integrity of these membranes. The activities of alkaline phosphatase and gamma-glutamyl transpeptidase, two membrane enzymes whose activities reflect the functional state of the hepatic membrane (Meister and Tate, 1976), are also restored following the treatment of CCl_4-intoxicated rats with silibinin (Muriel and Mourelle, 1990). Mourelle and Favari (1988) have also demonstrated that silibinin improves the metabolic disposition of aspirin (*e.g.*, deacetylation by plasma and tissue esterases) studied in rats with liver fibrosis induced by chronic treatment with CCl_4. An interesting feature of silibinin (and also of silymarin) is its regulatory action on the GSH content of different organs. When rats receive silymarin (ip) or silibinin (iv), significant increases in GSH contents of liver, intestine and stomach are observed. However, the tripeptide concentration in the lungs, spleen and kidneys remains unchanged (Valenzuela *et al*, 1989). This organ-specific action of the flavonoid may be due conceivably to differences in

those metabolic characteristics of each organ related to GSH synthesis and turnover, and/or to specific effects of silibinin upon hepatic, gastric and intestinal cells (Valenzuela *et al*, 1989). Pharmacokinetic studies of silymarin show that the plasma half-life of the flavonoid (in human and rats) is relatively short, being the liver the main target organ (Mennicke, 1976), where silymarin accumulates (Vogel and Trost, 1975). Over 80% of silymarin and its metabolites are excreted by the biliary tract as glucuronide and sulfoglucuronide conjugates (Lorenz *et al*, 1984). The flavonoid initiates a cyclic transportation via entero-hepatic circulation, because the gut flora splits the silymarin conjugates (Vogel and Trost, 1975). As result of this, the liver, the stomach and the intestine are the tissues bearing the higher silymarin concentrations, and also the tissues where the flavonoid induces large increases in GSH concentrations.

III. EFFECT OF SILIBININ AT NUCLEAR LEVEL

In addition to the antilipoperoxidative and GSH sparing effects described for silibinin, Machicao and Sonnenbichler (1977) and Sonnenbichler *et al* (1980), have carried out a series of experimental protocols demonstrating several effects of silibinin at the nuclear level. Silibinin increases the synthetic rate of the ribosomal RNA species 5.8S, 18S and 28S by about 20% (Sonnenbichler and Zetl, 1984). This stimulation was observed in rat liver, hepatocyte cultures and in isolated liver nuclei via activation of the DNA-dependent RNA polymerase I (Sonnenbichler and Zetl, 1985). Subsequently, the formation of mature ribosomes is stimulated and, as an important consequence, the protein biosynthesis in the liver is increased as well (Sonnenbichler and Zetl, 1985). Sonnenbichler *et al* (1986) have also demonstrated that the flavonoid increases DNA replication in the liver of rats partially hepatectomized. This effect evidences the liver cell regenerating capacity ascribed to the flavonolignane derivative (Hahn *et al*, 1968), supporting the clinical reports on this subject (Fintelmann and Albert, 1980). Stimulation of ribosomal RNA synthesis and DNA replication appear to be specific for silibinin, because the flavonoid shows a larger stimulatory effect than 35 other flavonoid derivatives tested (Sonnenbichler and Zetl, 1988).

By comparing the structure of different flavonoids, using space filling atomic models, it has been proposed that these natural vegetable substances have some similarities to sterols (Sonnenbichler and Zetl, 1988). Silibinin can compete specifically with the estradiol receptor site at concentrations higher than 2×10^{-7} M, which means a somewhat reduced affinity of the flavonolignane as compared to the pure hormone (Sonnenbichler and Zetl, 1988). The kinetics of the influence of estradiol on ribosomal RNA synthesis is indeed similar to that of silibinin (Sonnenbichler and Zetl, 1988). To explain this, it has been hypothesized that silibinin might interact as a regulator directly with polymerase I (Sonnenbichler and Zetl, 1985). One of the subunits of the enzyme might bear a receptor site for the steroid hormone which could be substituted by silibinin (Sonnenbichler and Zetl, 1988). As stated before, it is known from clinical findings that silibinin enhances the regenerative capacity of liver tissue after intoxication (Fintelmann and Albert, 1980). From the biochemical point of view, regeneration represents an increase in protein synthesis, which has been demonstrated for silibinin, but also an increase in proliferation and DNA synthesis. Protein and RNA syntheses are prerequisites for DNA synthesis. Therefore, it seems reasonable that DNA synthesis might also be influenced by the flavolignane derivative, as stated by Sonnenbichler and Zetl (1986).

FINAL CONSIDERATIONS

Although the biochemical mechanism(s) of action of the flavonoid silymarin (or silibinin) is (are) not yet well understood, some considerations may be drawn from the above description. The "hepatoprotective action" of the flavonoid may be mainly ascribed to its free radical scavenger properties. This effect is reflected in the membrane stabilizing and GSH-sparing actions described for the

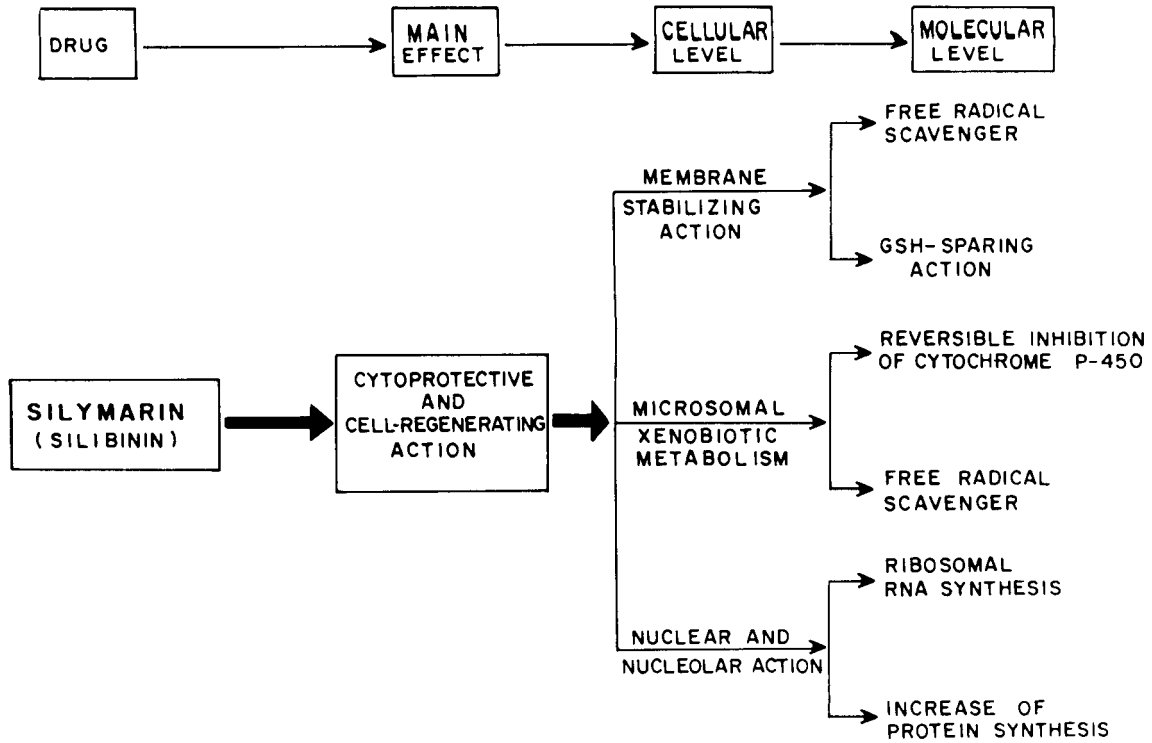

Fig 2. Proposed main actions of silymarin at cellular and molecular levels.

flavonoid, thus providing effective protection against the toxicity induced by a number of xenobiotics. The flavonoid may also act at the nuclear level, enhancing the synthesis of ribosomal RNA and the cellular regeneration. The steroidal-like behaviour of the flavonoid on the control of DNA expression has also been proposed. Figure 2 summarizes the main proposed biochemical actions of silymarin.

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REFERENCES

BARBERINO F, SUCIN A, COTTUTIN C, BAN A (1977) Die wirkung von silymarin auf die experimentelle galaktosaminhepatitis bei der ratte. Wien Clin Wschr 89: 90-95

BERETZ A, CAZENAVE J, ANTON R (1982) Inhibition of aggregation and secretion of human platelets by quercetin and other flavonoids. Structure-activity relationships. Agents Actions 12: 382-387

BEYELER S, TESTA B, PERRISSOUD D (1988) Flavonoids as inhibitors of rat liver monooxygenase activities. Biochem Pharmacol 37: 1971-1978

BINDOLI A, CAVALLINI L, SILIPRANDI N (1977) Inhibitory action of silymarin of lipid peroxide formation in the rat liver mitochondria and microsomes. Biochem Pharmacol 26: 2405-2409

BLACK M (1980) Acetaminophen hepatotoxicity. Gastroenterology 78: 382-392

CAMPOS R, GARRIDO A, GUERRA R, VALENZUELA A (1988) Silybin dihemisuccinate protects against glutathione depletion and lipid peroxidation induced by acetaminophen on rat liver. In: CODY V, MIDDLETON E, HARBORNE J, BERETZ A (eds) Plant Flavonoids in Biology and Medicine: Biochemical, Cellular and Medicinal Properties. New York: Alan R Liss. pp 275-378

CAMPOS R, GARRIDO A, GUERRA R, VALENZUELA A (1989) Silybin dihemisuccinate protects against glutathione depletion and lipid peroxidation induced by acetaminophen on rat liver. Planta Med 55: 417-419

COMPORTI M (1985) Biology of disease: Lipid peroxidation and cellular damage in toxic liver injury. Lab Invest 53: 599-623

CORCORAN G B, MITCHELL J R, VAISHNAV Y N (1980) Evidence that acetaminophen and N-hydroxy acetaminophen form a common arylating intermediate, N-acetyl-p-benzoquinone imide. Mol Pharmacol 18: 536-549

CHOPPIN J, DESPLACES A (1979) The action of silybin on the mouse liver in alpha-amanitine poisoning. Arzneim Forsch 29: 63-68

DUBIN M, GROSZMANN RJ, KRAVETZ D, COSTA JA, CANTORY D (1976) Acción de la silimarina sobre la hepatotoxicidad inducida por tetracloruro de carbono en ratas. Medicina, Buenos Aires 36: 437-442

DuBOIS PR, HILL RK, BURK RF (1983) Antioxidant effect of acetaminophen in rat liver. Biochem Pharmacol 32: 2621-2622

FINTELMANN V, ALBERT A (1980) Nachweis der therapeutischen Wirksamkeit von Legalon bei toxischen Lebererkrankungen im Doppelblindversuch. Therapiewoche 30: 5589-5594

FRAGA C, MARTINO V, FERRARO G, COUSSIO J, BOVERIS A (1987) Flavonoids as antioxidants evaluated by *in vitro* and *in situ* liver chemiluminescence. Biochem Pharmacol 36: 717-720

GARRIDO A, GUERRA R, VALENZUELA A (1988) The flavonoid silymarin increases the permeability of rat bone marrow cells to (^{3}H)-uridine. Res Commun Chem Pathol Pharmacol 61: 273-276

GARRIDO A, FAIRLIE J, GUERRA R, CAMPOS R, VALENZUELA A (1989) The flavonoid silybin ameliorates the protective effect of ethanol on acetaminophen hepatotoxicity. Res Commun Subst Abuse 10: 193-196

GARRIDO A, ARANCIBIA C, CAMPOS R, VALENZUELA A (1991) Acetaminophen does not induce oxidative stress to isolated rat hepatocytes : Its probably antioxidant effect is potentiated by the flavonoid silybin. Pharmacol Toxicol 69: 9-12

GUENGERICH P (1990) Enzymatic oxidation of xenobiotic chemicals. CRC Biochem Mol Biol 25: 97-153

HAHN G, LEHMAN H, KURTEN M, UEBEL H, VOGEL G (1968) Zur Pharmakologie und Toxikologie von Silymarin, des antihepatotoxischen Wirkprizips aus *Silybum marianun* (L) Gaerth. Arzneim Forsch 18:698-702

HAVSTEEN B (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 32: 1141-1148

JANIAK B (1974) Depression of microsomal activity in the liver of mice following single administration of halothane and its influenceability by silybin. Anaesthetist 23: 389-393

KOCH H, TSCHERNY J (1983) Splitting of silibyn dihemisuccinate by plasma and liver esterases. Arch Pharm 316: 426-430

KOCH H, DEMETER T, ZINSBERGER G (1980) Physikochemische Eigenschaften, 1. Mitt. pKa und Ionisationsprofil von Silybin, Silydianin und Silychristin. Arch Pharm 313: 565-571

LECOMTE J (1975a) Propriétés pharmacologiques générales de la silybine et de la silymarine chez le rat. Arch Intl Pharmacodyn Thér 214: 165-176

LECOMTE J (1975b) Les propriétés pharmacologiques de la silybine et de la silymarine. Rev Med Liège 30: 110-114

LORENZ D, LUCKER P W, MENNICKE W, WETZELSBERGER N (1984) Pharmacokinetic studies with silymarin in human serum and bile. Meth Find Exp Clin Pharmacol 6: 655-661

MACHICAO F, SONNENBICHLER J (1977) Mechanism of the stimulation of RNA synthesis in rat liver nuclei by silybin. Hoppe-Seyler's Z Physiol Chem 358: 141-147

MASON R, FISCHER V (1986) Free radicals of acetaminophen: Their subsequent reactions and toxicological significance. Fed Proc 45: 2493-2499

MEISTER A, TATE S (1976) Glutathione and related gamma-glutamyl compounds: Biosynthesis and utilization. Annu Rev Biochem 45: 559-609

MENNICKE W (1976) Pharmacokinetics of silymarin in humans and rats. In: BRAATZ R, SCHNEIDER C (eds) Symposium on the Pharmacodynamics of Silymarin. München: Urban & Schwarzenberg. pp 48-101

MIDDLETON E (1984) The flavonoids. Trends Pharmacol Sci 5: 335-338

MINOTTI G (1989) Ter-butyl hydroperoxide-dependent microsomal release of iron and lipid peroxidation. Arch Biochem Biophys 273: 137-143

MITCHELL JR, JOLLOW DJ, POTTER WZ, DAVIES DC, GILLETTE JR, BRODIE BB (1973) Acetaminophen-induced hepatic necrosis. 1. Role of drug metabolism. J Pharmacol Exp Ther 187: 185-194

MOURELLE M, FAVARI L (1988) Silymarin improves metabolism and disposition of aspirin in cirrhotic rats. Life Sci 43: 201-207

MOURELLE M, MURIEL P, FAVARI L, FRANCO T (1989) Prevention of CCl4-induced liver cirrhosis by silymarin. Fundam Clin Pharmacol 3: 183-191

MURIEL P, MOURELLE M (1990) Prevention by silymarin of membrane alterations in acute CCl_4 liver damage. J Appl Toxicol 10: 275-279

MURIEL P, GARCIAPINA T, PEREZ-ALVAREZ V, MOURELLE M (1994) Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J Appl Toxicol (In press)

NIETO S, GARRIDO A, SANHUEZA J, LOYOLA L, MORALES G, LEIGHTON F, VALENZUELA A (1993) Flavonoids as stabilizers of fish oil: An alternative to synthetic antioxidants. J Am Oil Chem Soc 70: 773-778

PELTER A, HANSEL R (1968) The structure of silybin (silybum substance E6), the first flavonolignan. Tetrahedron Lett 25: 2911-2916

POTTER D, HINSON J (1987) Mechanisms of acetaminophen oxidation to n-acetyl-p-benzoquinone imine by horseradish peroxidase and cytochrome P-450. J Biol Chem 262: 966-973

SCHRIEWER H, RAUEN M (1971) Die antihepatotoxische Wirkung von parenteral verabreichtem Silymarin bei der Galaktosamin Hepatitis der Ratte. Arzneim Forsch 21: 1194-1198

SCHRIEWER H, BADDE R, ROTH G, RAUEN HM (1973) Die pharmakokinetik der antihepatotoxischen Wirkung des Silymarins bei der Leberschadigung der Ratte durch CCl_4 und Desoxycholat. Arzneim Forsch 23: 160-161

SIES H (1986) Biochemistry of oxidative stress. Angew Chem 25: 1058-1071

SONNENBICHLER J, ZETL I (1984) Influence of silibinin on the synthesis of ribosomal RNA, mRNA and tRNA in rat livers *in vivo*. Hoppe-Seyler's Z Physiol Chem 365: 555-566

SONNENBICHLER J, ZETL I (1985) Influence of the flavonolignan derivative silibinin on nuclei acid and protein synthesis in liver cells. In: FARKAS L, GABOR M, KALLAY F (eds) Flavonoids and Bioflavonoids. New York: Alan R Liss. pp 361-372

SONNENBICHLER J, ZETL I (1986) Biochemical effects of the flavonolignane silibinin on RNA, protein and DNA synthesis in rat livers. In: CODY V, HARBORNE J (eds) Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships. New York: Alan R Liss. pp 319-331

SONNENBICHLER J, ZETL I (1988) Specific binding of a flavonolignane derivative to an estradiol receptor. In: CODY V, MIDDLETON E, HARBORNE J, BERETZ A (eds) Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties. New York: Alan R Liss. pp 369-374

SONNENBICHLER J, MATTERSBERGER J, HANSER G (1980) Investigations of the mechanism of action of silybin III. Uptake of the flavonolignane silybin by the rat liver cells. Hoppe-Seyler's Z Physiol Chem 361: 1751-1756

SONNENBICHLER J, GOLDBERG M, HANE L, MADUBUNYL I, VOGL S, ZETL I (1986) Stimulatory effect of silibinin on the DNA synthesis in partially hepatectomized rat livers: non-response in hepatoma and other malign cell lines. Biochem Pharmacol 35: 538-541

STOCKINGER L, TROST W, UEBEL H (1976) Quantification électronique des lésions hépatiques experimentales, mises en évidence en histologie. Arch Anat Cytol Pathol 24: 203-209

SVINGEN BA, BUEGE JA, O'NEAL FO, AUST SD (1979) The mechanism of NADPH-dependent lipid peroxidation. The propagation of lipid peroxidation. J Biol Chem 254: 5892-5899

TSOKOS-KUHN J, HUGHES H, SMITH C, MITCHELL J (1988) Alkylation of the liver plasma membrane and inhibition of the Ca^{2+} ATPase by acetaminophen. Biochem Pharmacol 37: 2125-2131

VALENZUELA A, GUERRA R (1985) Protective effect of the flavonoid silybin dihemisuccinate on the toxicity of phenylhydrazine on rat liver. FEBS Lett 181: 284-291

VALENZUELA A, GUERRA R (1986) Differential effect of silybin on the Fe^{2+}-ADP and t-butyl hydroperoxide-induced microsomal lipid peroxidation. Experientia 42: 139-141

VALENZUELA A, RIOS H, NEIMAN G (1977) Superoxide radicals and the hemolytic mechanism of phenylhydrazine. Experientia 33: 962-963

VALENZUELA A, FERNANDEZ V, FERNANDEZ N, UGARTE G, VIDELA LA (1980) Effect of acute ethanol ingestion on lipoperoxidation and on the activity of enzymes related to peroxide metabolism. FEBS Lett 111: 11-14

VALENZUELA A, BARRIA T, GUERRA R, GARRIDO A (1985a) Inhibitory effect of the flavonoid silymarin on the erythrocyte hemolysis induced by phenylhydrazine. Biochem Biophys Res Commun 126: 712-716

VALENZUELA A, LAGOS C, SCHMIDT K, VIDELA LA (1985b) Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat. Biochem Pharmacol 34: 2209-2212

VALENZUELA A, GUERRA R, VIDELA LA (1986) Antioxidant properties of the flavonoids silybin and (+)-cyanidanol-3: Comparison with butylated hydroxy anisole and butylated hydroxy toluene. Planta Med 6: 438-440

VALENZUELA A, GUERRA R, GARRIDO A (1987) Silybin dihemisuccinate protects rat erythrocytes against phenylhydrazine-induced lipid peroxidation and hemolysis. Planta Med 53: 402-405

VALENZUELA A, ASPILLAGA M, VIAL S, GUERRA R (1989) Selectivity of silymarin on the increase of the glutathione content of different tissues of the rat. Planta Med 55: 420-422

VIDELA LA, VALENZUELA A (1982) Minireview: Alcohol ingestion,liver glutathione and lipoperoxidation; metabolic interrelations and pathological implications. Life Sci 31: 2395-2399

VIDELA LA, FERNANDEZ V, UGARTE G, VALENZUELA A (1980) Effect of acute ethanol intoxication on the content of reduced glutathione of the liver in relation to its lipoperoxidative capacity in the rat. FEBS Lett 111: 6-10

VIDELA LA, FERNANDEZ V, de MARINIS A, FERNANDEZ N, VALENZUELA A (1982) Liver lipoperoxidative pressure and glutathione status following acetaldehyde and aliphatic alcohols pretreatment in the rat. Biochem Biophys Res Commun 104: 945-949

VLADUTIU GD, MIDDLETON E (1986) Effects of flavonoids on enzyme secretion and endocytosis in normal and mucolipidosis II fibroblasts. Life Sci 39: 717-726

VOGEL G (1968) Silymarin, das antihepatotoxische Wirskprinzip aus *Silybum marianum* (L), Gaerth. Als antagonist der phalloidin-wirkung. Arzneim Forsch 18: 1063-1064

VOGEL G (1981) The anti-amanita effect of silymarin. In: FAULSTICH H, KOMMERELL B (eds) Amanita Toxins and Amanita Poisoning. New York: Witzstrock Publ. pp 315-322

VOGEL G, TROST W (1975) Zur anti-phalloidin-aktivitat der silymarin, silybin und disilybin. Arzneim Forsch 25: 392-393

VOGEL G, TROST W, MENGS U (1977) Counteraction of amanitin-induced liver damage in rats by silybin. Naunyn-Schmiedeberg's Arch Pharmakol 19: 297-281

WAGNER H, HORHAMMER L, MUNSTER R (1965) Ube die Struktur eines neuen Flavonoids aus den Fruchten von *Carduus marianus* L. Naturwissenschaften 52: 305-308

WAGNER H, DIESEL P, SEITZ M (1974) Zur chemie und analytik von silymarin aus *Silybum marianum*, Gaerth. Arzneim Forsch 24: 466-470

WENDEL A, FEUERSTEIN S, KONZ HK (1979) Acute paracetamol intoxication of starved mice leads to lipid peroxidation *in vivo*. Biochem Pharmacol 28: 2051-2055

WEISS S, LoBUGLIO A (1982) Biology of disease: Phagocyte-generated oxygen metabolites and cellular injury. Lab Invest 47: 5-18