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Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver

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Summary

Silymarin, the active principle of the milk thistle *Silybum marianum*, protects experimental animals against various hepatotoxic substances. To determine the effect of silymarin on the outcome of patients with cirrhosis, a double blind, prospective, randomized study was performed in 170 patients with cirrhosis. 87 patients (alcoholic 46, non-alcoholic 41; 61 male, 26 female; Child A, 47; B, 37; C, 3; mean age 57) received 140 mg silymarin three times daily. 83 patients (alcoholic 45, non-alcoholic 38; 62 male, 21 female; Child A, 42; B, 32; C, 9: mean age 58) received a placebo. Non-compliant patients and patients who failed to come to a control were considered as 'drop outs' and were withdrawn from the study. All patients received the same treatment until the last patient entered had finished 2-years of treatment. The mean observation period was 41 months. There were 10 drop outs in the placebo group and 14 in the treatment group. In the placebo group, 37 (+2 drop outs) patients had died, and in 31 of these, death was related to liver disease. In the treatment group, 24 (+4 drop outs) had died, and in 18 of these, death was related to liver disease. The 4-year survival rate was 58 ± 9% (S.E.) in silymarin-treated patients and 39 ± 9% in the placebo group ($P = 0.036$). Analysis of subgroups indicated that treatment was effective in patients with alcoholic cirrhosis ($P = 0.01$) and in patients initially rated 'Child A' ($P = 0.03$). No side effects of drug treatment were observed. The results of this study suggest that mortality of patients with cirrhosis was reduced by treatment with silymarin. However, as this effect was more pronounced in alcoholic cirrhosis, the interrelation of patterns of alcohol consumption and of drug treatment affecting survival must be adressed by future studies.

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Introduction

Silymarin is the collective name for the flavolignans silibinin, silidianin and silichristin, extracted from the milk thistle *Silybum marianum* (L.) Gartneri. This drug was shown to protect experimental animals against various hepatotoxins including phalloidin [1,2], α-amanitin [3], carbontetrachloride [4,5], thioacetamide [6] and galactosamine [4]. The mechanism by which silymarin exerts its 'hepatoprotective' action is under intensive investigation. The antitoxic effects of silymarin against the *Amanita phalloides* toxins phalloidin and α-amanitin are mediated by the inhibition of the binding of phalloidin to specific receptors on liver cell membranes [7] and by the antagonism of the blocking effects of α-amanitin on RNA-polymerase [8–10]. Furthermore, the 'hepatoprotective' properties of silymarin can be related to the inhibition of lipid peroxide formation in liver cells [11] and to changes in the physical properties of plasma membranes induced by silymarin, making cells more resistant to osmotic lysis and to the action of detergents [12,13].

The role of silymarin for treatment of patients with liver disease remains to be established. The intravenous administration of silibinin-C-2',3-dihydrogen succinate, the water-soluble active form of silymarin, prevents hepatocelluiar disease when given to patients within the first hours after the ingestion of *Amanita phalloides* [2]. In patients with mild alcoholic liver disease receiving silymarin, abnormal liver 'function' tests improved more rapidly than in those receiving a placebo [14,15]. This prospective double blind randomized study was initiated to determine whether silymarin improves the prognosis of patients with cirrhosis of the liver.

Patients and study protocol

170 patients with cirrhosis of the liver were included in the study. Diagnosis of cirrhosis was made within 2 years before entering the study. Two thirds of the patients were newly diagnosed cases. In 70% of the patients, diagnosis of cirrhosis was confirmed by liver histology. In the remaining patients, no liver biopsy could be obtained due to coagulation disorders. Patients were recruited from all patients with cirrhosis seen at one of the four participating centers (1st Department of Gastroenterology and Hepatology, University of Vienna, Departments of Internal Medicine, Sophienspital and Krankenhaus Floridsdorf, Vienna, and Outpatient Clinic 'South', Vienna Health Insurance). Patients with end-stage liver failure and patients with known malignancies were not considered for the study. Patients on immunosuppressive treatment and patients with primary biliary cirrhosis were excluded. All potential candidates were followed for at least 3 months before they were asked to participate. By this approach, very sick patients and likely non-compliant patients were excluded. More than 90% of the eligible patients agreed to participate in the study.

At entry into the study, the severity of the underlying liver disease was classified using the Child-Turcotte criteria [16] (Child A, 5–7; Child B, 8–12; Child C, 13–15 points) and the etiology using clinical, biochemical, immunological and histological criteria as described previously [17]. The patients were assigned according to a random-number sequence to receive either 420 mg of silymarin (140 mg three times/day orally) or a placebo of identical appearance. This dose of silymarin was based on pharmacokinetic studies in the hope of achieving hepatic concentrations of the drug similar to those shown to be effective in in vitro studies [18]. Both the drug and the placebo were kindly supplied by Madaus & Co., Köln, F.R.G. All patients were advised to abstain from alcohol completely. The degree of alcohol abuse during the study was estimated by questioning the patients and their relatives on the amount of alcohol consumed. Furthermore, serial determinations of serum γ-glutamyl-transpeptidase (SGGTP) activity in the blood were a useful parameter to detect major alcohol abuse during the study. Any therapy of complications of liver disease or of other conditions unrelated to liver disease was recorded in the protocol. The use of steroids and of D-penicillamine was not allowed.

After randomization, the patients were seen at

three monthly intervals. At each out-patient visit, the patient was examined and blood was drawn to determine routine parameters of liver function (bilirubin, SGOT, SGPT, alkaline phosphatase, SGGPT, prothrombin time, albumin, pseudocholinesterase, serum electrophoresis).

At the beginning of the study and at each following visit to the out-patient clinic, the patients received a 3 month supply of the drug and were asked to return the containers at the next scheduled visit. Counting the unused capsules served as a measure for compliance with treatment. Patients were withdrawn from the study and considered as 'drop outs' if they failed to come to a scheduled out-patients visit within 14 days or if they had not used at least 80% of the capsules supplied. If a patient died during the study, the physician treating the final event was contacted to determine the cause of death.

Originally, the study was designed to last for 2 years. Later, as recruitment of patients for the study took considerably longer than originally assumed, it was decided by the study committee to extend the study until the last patient entered had completed the 2-year study period. Each patient who completed the 2-year study period was asked to continue the treatment without breaking the code. The mean duration of treatment was 41 months (range, 2–6 years).

Statistical analysis

The data were evaluated by a computer using the life table and survival function analysis (BMDP Statistical Software, Inc., Regents of the University of California, 1983). Using this program the product limit estimate of survival was computed. Statistical comparison of the results was performed using a general Wilcoxon-Breslov test and the Mantel-Cox test [19]. The Mantel-Cox test weights mortality equally during the whole study, the Wilcoxon-Breslov test is more sensitive in the early phase of survival. The χ^2-test was used for comparison of demographic data and to compare mortality figures after 2 years and the unpaired Student's *t*-test for comparison of biochemical parameters of liver function between the study groups.

Results

83 patients received placebo and 87 were treated with silymarin. The two study groups were well matched regarding etiology and severity of the underlying liver disease, age, sex and biochemical findings (see Tables 1 and 2). Liver histology showed inactive cirrhosis or mildly active cirrhosis in 91 patients (49 in the treatment group and 42 in the control

TABLE 1

DEMOGRAPHIC DATA OF PATIENTS WITH CIRRHOSIS OF THE LIVER IN THE SILYMARIN TRIAL AT RANDOMIZATION

	Placebo	Silymarin
Male (*n*)	62	61
Female (*n*)	21	26
Age (years, mean ± S.D.)	58 ± 12	57 ± 12
Etiology of liver disease		
Alcoholic (*n*)	45	47
Non-alcoholic (*n*)	38	40
Ascites	26	21
Child classification		
A (*n*) (alcoholic/non-alcoholic)	42 (18:24)	47 (26:21)
B (*n*) (alcoholic/non-alcoholic)	32 (21:11)	37 (18:19)
C (*n*) (alcoholic/non-alcoholic)	9 (6:3)	3 (3:0)

TABLE 2

BIOCHEMICAL DATA OF PATIENTS WITH CIRRHOSIS OF THE LIVER IN THE SILYMARIN TRIAL AT RANDOMIZATION

	Placebo	Silymarin
Serum-bilirubin (mg/dl)	2.5 ± 2.1	1.6 ± 1.1
SGOT (U/l)	40 ± 38	36 ± 30
SGPT (U/l)	28 ± 33	29 ± 22
SGGTP (U/l)	150 ± 178	129 ± 160
Serum-albumin (g/l)	35.6 ± 6.6	35.8 ± 6.0
Prothrombin time (%)	67 ± 22	69 ± 17
HbsAg positive (*n*)	12	9

SGOT = serum glutamic oxaloacetic transaminase (normal up to 12 U/l); SGPT = serum glutamic pyruvic transaminase (normal up to 12 U/l); SGGTP = serum gamma-glutamyl transpeptidase (normal up to 28 U/l). Normal range for serum albumin, 35–50 g/l; normal range for prothrombin time, 70–130%. Data are shown as mean ± S.D.

group) and cirrhosis with alcoholic hepatitis in 28 (16 in the treatment group and 12 in the control group). No biopsy could be obtained in 22 and 29 patients in the treatment and control group, respectively.

Silymarin treatment was well tolerated over the whole study period. Only 4 patients (2 in the silymarin and 2 in the control group) complained of nausea and epigastric discomfort. These complaints disappeared after stopping the drug treatment.

Ten patients in the placebo (3 non-alcoholics and 7 alcoholics) and 14 patients in the silymarin group (7 alcoholics and 7 non-alcoholics) were considered as 'drop outs'. The reasons for 'drop out' were drug-related complaints (see above, 2 in each group), intercurrent major non-hepatic disease (1 in each group), change of the treating physician due to moving house (1 in the control and 2 in the silymarin group). In the remaining 15 patients no clear reasons for drop out were apparent ('true non-compliance' i.e., patients not taking the tablets and/or not showing up for control examinations). In the life table analysis, the 'drop outs' were considered as 'withdrawn' at the date of their last regular control in the study. The fate of these patients further was observed within post-study sulveillance. The results of this surveillance beyond the 2-year study period will be published later.

105 patients completed the 2-year study period (48 in the control and 57 in the treatment group). All 94 patients in whom the 2-year study period was terminated more than 3 months before the last one, agreed to continue the study. 43 were studied for 3–4 years and 29 for 4 or more years. The median duration of treatment was 41 months. Within the 2-year study period, mortality was 33% (27 out of 83) and 23% (20 out of 87) ($P = 0.07$) in the placebo and silymarin group, respectively. By life table analysis, the cumulative 2-year survival rate was 82 ± 4% (± S.E.) and 68 ± 5% in the treatment and control group, respectively. During the whole study, an additional 12 patients in the placebo group and 8 in the treatment group had died. The causes of death are listed in Table 3. 32 patients (21 in the control and 11 in the silymarin group) developed end-stage liver failure and died. 11 patients without overt signs of end stage liver failure died following massive upper gastrointestinal hemorrhage (9 due to rupture of esophageal varices and 2 to a bleeding duodenal ulcer). 1 patient developed a hepatocellular carcinoma 4.5 years after randomization and died shortly after diagnosis. In 12 patiens the cause of death was unrelated to liver disease. In 5 patients the cause of death could not be determined with confidence: one of them collapsed

TABLE 3

CAUSES OF DEATH OF PATIENTS DYING DURING THE SILYMARIN STUDY

	Alcoholic		Non-alcoholic		Drop outs		All patients	
	P	S	P	S	P	S	P	S
End-stage liver failure	11	4	10	7	2	2	23	13
Upper GI hemorrhage	3	2	5	1	–	–	8	3
Hepatocellular carcinoma	–	–	1	–	–	–	1	–
Non-hepatic malignancies	2	–	1	1	–	1	3	2
Accident	1	1	–	–	–	–	1	1
Heart failure	1	1	–	1	–	–	1	2
Cerebral hemorrhage	1	–	–	–	–	1	1	1
Pneumonia	–	1	–	–	–	–	–	1
Meningitis	–	1	–	–	–	–	–	1
Unknown	–	–	1	4	–	–	1	4
Total	19	10	18	14	2	4	39	28

P = placebo; S = silymarin.

while walking on the street and died. The 4 remaining patients were found dead in their homes. 59 of the 67 dead patients underwent autopsy. At the postmortem examination in 8 (5 in the control and 3 in the silymarin group), a clinically unsuspected small hepatocellular carcinoma was found. Of the drop outs, 4 died of end-stage liver failure (2 in each group), one following stroke and on due to hypernephroma (both in the treatment group); 18 were alive 2 years after randomization.

Fig. 1 shows the life table analysis of both groups. After 4 years, the cumulative survival was 58 ± 9% and 38 ± 9% in the treatment group and in the control group, respectively ($P = 0.036$). In addition, survival rates were separately analysed according to the etiology of the liver disease (alcoholic versus non-alcoholic) and according to the severity of liver disease (see Figs. 2 and 3). In alcoholic cirrhosis, the number of deaths in the control group (19) was twice that of the treatment group (10) and treatment was associated with a better outcome ($P = 0.01$). Conversely, in non-alcoholic cirrhotics, the survival rates were not significantly influenced by treatment with silymarin. In patients originally rated Child A, survival was significantly improved by treatment with silymarin ($P = 0.03$). In patients originally rated Child B or C, survival rates did not differ in the two groups.

Analysis of liver function tests, such as transaminases, pseudocholinesterase, SGGPT, bilirubin and alkaline phosphatase revealed no difference between the two study groups. As judged by SGGTP determinations, 33 patients in the control group and 26 patients in the treatment group consumed alcohol during the study.

Discussion

This study demonstrates that long-term treatment with silymarin reduced the mortality of patients with

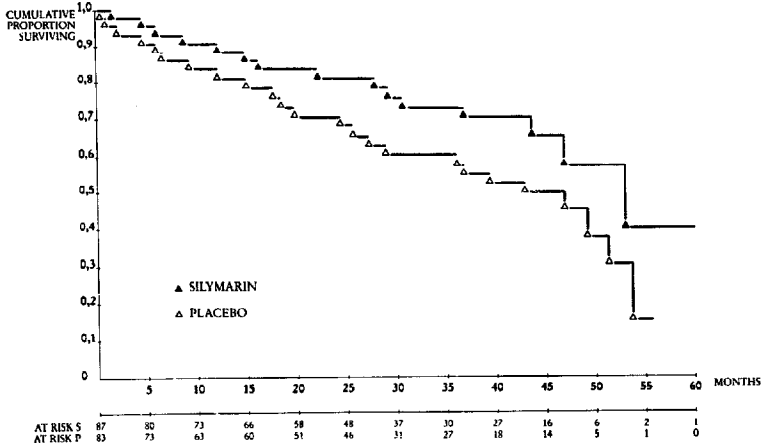

Fig. 1. Survival curves for 170 patients with cirrhosis of the liver treated with silymarin or placebo (Kaplan-Meier Life-Table analysis, Wilcoxon-Breslow test $P = 0.036$; Mantel-Cox test $P = 0.058$).

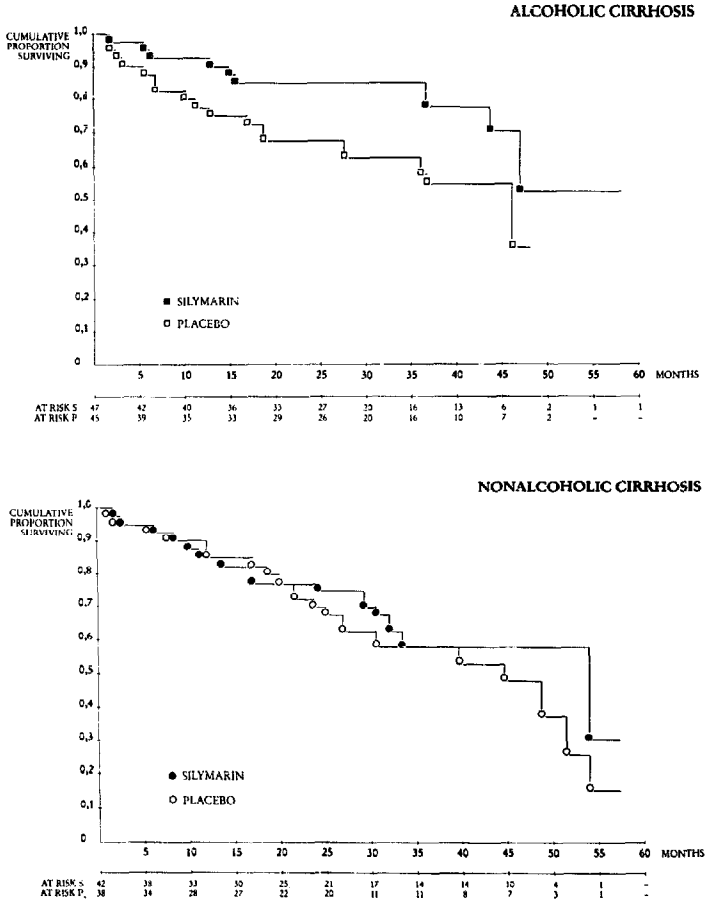

Fig. 2. Survival curves for 170 patients with cirrhosis of the liver treated with silymarin or placebo; data analysed according to the etiology of liver disease (placebo vs. silymarin, alcoholic cirrhosis: Wilcoxon-Breslov test $P = 0.011$; Mantel-Cox test $P = 0.012$; non-alcoholic cirrhosis: Wilcoxon-Breslov test $P = 0.72$; Mantel-Cox test $P = 0.98$).

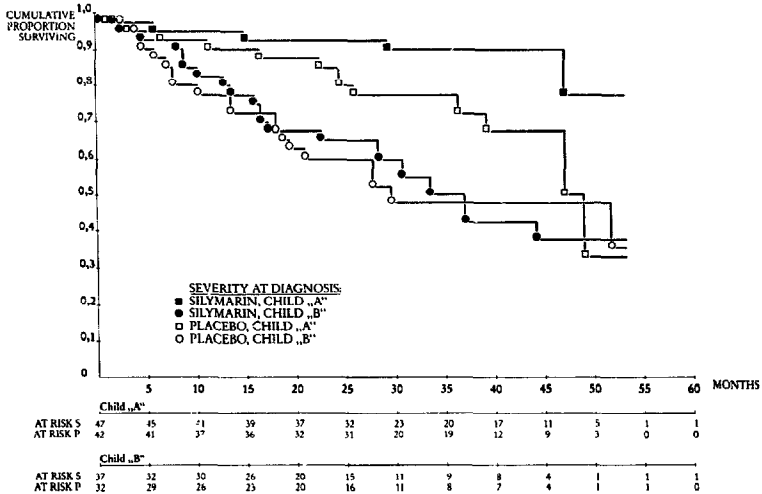

Fig. 3. Survival curves for 170 patients with cirrhosis of the liver treated with silymarin o: placebo; data anlysed according to the severity of liver disease at randomization (placebo vs. silymarin: Child A, Wilcoxon-Breslov test $P = 0.031$; Mantel-Cox test $P = 0.016$; Child B, Wilcoxon-Breslov test $P = 0.65$; Mantel-Cox test $P = 0.86$).

cirrhosis. While this conclusion is well supported by the statistical analysis of the data, caution has to be applied when interpreting the findings. First, although no significant differences were observed between the two study groups regarding demographic and disease-related characteristics, patients in the placebo group were slightly sicker than those in the treatment group as shown by higher mean bilirubin levels and a greater number of 'Child C' patients. Although these differences were not significant ($P > 0.3$) they may have influenced the outcome of this study. Second, the degree of alcohol consumption during the study has to be considered, as the survival of patients becoming abstinent is greater than of those who continue to drink [22]. There are no accepted objective and reliable criteria to detect alcohol abuse. Self-reporting has been shown to be unreliable [23]. Serial determinations of SGGTP were used in this study to monitor for alcohol abuse and helped to identify some of the patients drinking alcohol. However, this test may not be sufficient to exclude ongoing alcohol abuse, since SGGTP levels do not correlate with the duration of alcohol abuse or the daily quantity of alcohol consumed, and the sensitivity of SGGTP to detect alcohol abuse is low [24]. Although in the control group, the number of patients with increased SGGTP levels was slightly greater than in the treatment group, there is no obvious reason to believe that the degree and frequency of alcohol abuse was different between the two groups. Furthermore, there is no evidence that silymarin has some 'antabus'-like properties.

Evaluation of the efficacy of drug treatment in patients with liver cirrhosis is difficult for many reasons.

There are no reliable parameters to assess a beneficial effect of the drug on the progression of chronic liver disease. Mortality is possibly not the best parameter, but it is still the best one available in therapeutic trials in patients with liver cirrhosis [20]. Undoubtedly, mortality is determined not only by drug treatment but also by several other factors, which are unlikely to be influenced by any treatment. The value of serial liver biopsies or of laboratory parameters to assess the efficacy of treatment in patients with alcoholic cirrhosis has never been documented [21]. Furthermore, liver biopsies are subject to sampling error and cannot be obtained safely from patients with advanced liver disease. In this study, follow-up transaminases, serum albumin and serum bilirubin revealed no differences between the two study groups. However, it should be noted that in the control group twice as many patients had died of hepatic failure than in the treatment group. As these patients had highly abnormal laboratory findings, after their death the mean value for a certain parameter improved in the remaining patients. Thereby, differences between the two groups could have been missed.

In an attempt to identify subgroups of patients in whom treatment with silymarin was more effective, survival rates were separately analysed according to the etiology and to the severity of liver disease. The result of this analysis indicated that treatment was most effective in patients with alcoholic liver disease and in patients originally rated Child A. However, it should be stressed that such an analysis cannot substitute for a stratified prospective study. While the whole study group was well matched regarding several variables including etiology and severity of liver disease, this was not the case in some of the subgroups analysed. Although none of these differences in distribution were significant (such as the greater number of Child A patients with alcoholic cirrhosis in the treatment group than in the placebo group), they may have influenced the results of survival analysis in the subgroups.

A drug that protects the liver cell from toxic substances could diminish hepatocellular necrosis and could thereby delay or prevent the occurrence of hepatic failure. A reduction in mortality due to hepatic failure can, therefore, be attributed to the effect of drug treatment. If silymarin treatment reduced the extent of hepatocellular damage, it may also have reduced the degree of collagen deposition in the liver and therefore the extent of circulatory changes in the liver and in the portal system may have been minimized. Thus, the decreased mortality due to variceal hemorrhage may possibly be related to the effect of drug treatment.

The mechanisms of action of silymarin are uncertain. In all clinical studies performed so far, the beneficial effects of silymarin have been documented in alcoholic or toxic liver disease [14,14]. In this study, silymarin decreased mortality most markedly in patients with alcoholic cirrhosis. Although all patients denied alcohol intake during the study, the complete cessation of alcohol abuse in a group of chronic alcoholic patients is very unlikely. As judged by serial determinations of SGGTP activity in the blood, about 60% of the patients with alcoholic cirrhosis in each group still consumed appreciable quantities of alcohol. Thus, the effect of silymarin appears to be the prevention of some metabolic or toxic effects of alcohol on the liver.

References

1 Tuchweber B, Sieck R, Trost W. Prevention by silybinin of phalloidin induced acute hepatotoxicity. Toxicol Appl Pharmacol 1979; 51: 265–275.

2 Floersheim GL. Experimentelle Grundlagen zur Therapie von Vergiftungen durch den grünen Knollenblätterpilz (*Amanita phalloides*). Schweiz Med Wschr 1978; 108: 185–197.

3 Hahn G, Lehmann HD, Kürten M, Uebel H, Vogel G. Zur Pharmakologie und Toxikologie von Silymarin, des antihepatotoxischen Wirkprinzipes aus *Silybum marianum* (L) Gaertn. Arzneim Forsch 1968; 18: 698–704.

4 Rauen HM, Schriewer H. Die antihepatotoxische Wirkung von Silymarin bei experimentellen Leberschädigungen der Ratte durch Tetrachlorkohlenstoff, D-Galaktosamin und Allylalkohol. Arzneim Forsch 1971; 21: 1194–1212.

5 Rauen HM, Schriewer H. Die antihepatotoxische Wirkung

von parenteral verabreichtem Silymarin bei der Leberschädigung der Ratte durch CCl_4. Arzneim Forsch 1973; 23: 148–149.
6 Schriewer H, Badde R, Roth G, Rauen HM. Die antihepatotoxische Wirkung des Silymarins bei der Leberschädigung durch Thioacetamid. Arzneim Forsch 1973; 23: 160–161.
7 Lotter HL. Untersuchung der Struktur-Wirkungsbeziehung antihepatotoxischer Naturstoffe (Silybin-Antamanid) durch Röntgenstrukturanalyse. Z. Naturforsch 1984; 39c: 535.
8 Sonnenbichler J, Zetl I. Undersuchungen zum Wirkungsmechanismus von Silibinin, V. Einfluss von Silibinin auf die Synthese ribosomaler RNA, mRNA und tRNA in Rattenlebern in vivo. Hoppe Seyler's Z Physiol Chem 1984; 365: 555–560.
9 Machicao F, Sonnenbichler J. Mechanism of the stimulation of RNA synthesis in rat liver nuclei by silybin. Hoppe Seyler's Z Physiol Chem 1977; 358: 141–147.
10 Faulstich H. New aspects of *Amanita* poisoning. Klin Wschr 1979; 57: 1143–1152.
11 Bindoli A, Cavallini L, Siliprandi N. Inhibitory action of silymarin of lipid peroxide formation in rat liver mitochondria and microsomes. Biochem Pharmacol 1977; 26: 2405–2409.
12 Valenzuela A, Barria T, Guerra R, Garrido A. Inhibitory effect of the flavonoid silymarin on the erythrocyte hemolysis induced by phenylhydrazine. Biochem Biophys Res Commun 1985; 126: 712–715.
13 Ramellini G, Meldolesi J. Stabilization of isolated rat liver plasma membranes by treatment in vitro with silymarin. Arzneim Forsch 1974; 24: 806–808.
14 Salmi HA, Sarna S. Effect of silymarin on chemical, functional and morphological alterations of the liver. Scand J Gastroenterol 1982; 17: 517–520.
15 Fintelmann V, Albert A. Nachweis der therapeutischen Wirksamkeit von Legalon® bei toxischen Lebererkrankungen im Doppelblindversuch. Therapiewoche 1980; 30: 5589.
16 Child CG, Turcotte JG. Surgery and portal hypertension. In: Child CG, ed. The Liver and Portal Hypertension. Philadelphia: WB Saunders & Co, 1964; 50–56.
17 Ferenci P, Dragosics B, Marosi L, Kiss F. Relative incidence of primary liver cancer in cirrhosis in Austria. Etiological considerations. Liver 1984; 4: 7–14.
18 Bulles H, Bulles J, Krumbiegel G, Mennicke WH, Nitz D. Untersuchungen zur Verstoffwechselung und zur Ausscheidung von Silybin bei der Ratte. Drug Res 1975; 25: 902–905.
19 Cox DR. Regression models and life tables (with discussion). J R Stat Soc B 1972; 34: 187–220.
20 Preisig R, Problems in phase I and phase II trials with 'hepatoprotective' drugs. In: Conn HO, ed. International workshop on (+) cyanidanol-3 in diseases of the liver. International Congress and Symposium Series, No. 47. London: The Royal Society of Medicine and Academic Press 1981; 41–48.
21 Juhl E, Christensen E, Tygstrup N. The epidemiology of the gastrointestinal RCT. N Engl J Med 1977; 296: 20–22.
22 Powell WJ, Klatskin G. Duration of survival in patiens with Laennec's cirrhosis. Am J Med 1968; 44: 406–420.
23 Orrego H, Blake JE, Blendis LM, Kapur BM, Israel Y. Reliability of assessment of alcohol intake based on personal interviews in a liver clinic. Lancet 1979; ii: 1354–1356.
24 Moussavian SN, Becker RG, Piepmeyer JL, Mezey E, Bozian RC. Serum gamma-glutamyl transpeptidase and chronic alcoholism. Influence of alcohol ingestion and liver disease. Dig Dis Sci 1985; 30: 211–214.