

## HEPATOPROTECTIVE ACTIVITY OF SILYMARIN IN RATS TREATED WITH HIGH DOSES OF ACETYLSALICYLIC ACID OR NAPROXEN

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### ABSTRACT

Rats were treated with acetylsalicylic acid (150 mg/kg b.w. per os daily) or naproxen (125 mg/kg b.w. per os daily) for six weeks. Half of the rats received silymarin (17.5 mg/kg b.w. per os daily) in the last three weeks of the experiment. It was found that administration of acetylsalicylic acid led to signs of hepatic damage (an increase in serum bilirubin level, alanine aminotransferase and  $\gamma$ -glutamyl transpeptidase activity) while the concomitant administration of silymarin diminished the extent of the hepatic damage. Naproxen was shown to be less toxic than acetylsalicylic acid, and its toxicity was also reduced by silymarin. The obtained results suggest that silymarin be administered to patients undergoing long-term treatment with non-steroidal anti-inflammatory drugs in order to prevent hepatic damage, but further studies are needed to elaborate on the clinical aspects of silymarin treatment in those patients.

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**Key Words:** Acetylsalicylic acid, Naproxen, Silymarin, Liver function

### INTRODUCTION

Long-term treatment with non-steroidal anti-inflammatory agents is a necessity for many patients with rheumatic disease. These drugs produce several adverse reactions, including hepatic damage. Hepatotoxicity due to acetylsalicylic acid has been reported in a number of studies, and is known as "aspirin hepatitis".<sup>1-6</sup> Other non-steroidal anti-inflammatory drugs are believed to be less harmful to the liver as compared to acetylsalicylic acid, although clinical case reports indicate that in some patients, these drugs can cause signs and symptoms of hepatic damage.<sup>7,8</sup>

Experimental studies with hepatic slices cultured *in vitro* with non-steroidal anti-inflammatory drugs con-

firmed the ability of these drugs to damage liver cells directly.<sup>9,10</sup> The extension of the toxic effect varied significantly with the drug used. Acetylsalicylic acid was found to be most toxic while naproxen was shown to produce relatively slight changes in the hepatic slices.

In the recent decade, various drugs which facilitate the recovery of hepatocytes after toxic damage (e.g. due to alcohol consumption) or viral infection have been introduced into clinical practice. Silymarin, a mixture of flavonoids isolated from *Silybum marianum*, is widely used in the treatment of impaired hepatic function. The mechanism of the drug's action remains partially unknown. The present study was designed to evaluate the basic biochemical indices of hepatic function in rats treated with some non-steroidal anti-inflammatory drugs with and without the concomitant administration of silymarin.

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## Silymarin Hepatoprotection in Rats

Table I. Indices of hepatic function in rats treated with acetylsalicylic acid or naproxen with or without silymarin

Group of animals	Bilirubin ( $\mu\text{mol/L}$ )	Alanine aminotransferase (nmol/L/s)	$\gamma$ -glutamyl transpeptidase (nmol/L/s)	Total protein (g/L)
Controls	5.95 $\pm$ 0.53	279.1 $\pm$ 29.5	17.17 $\pm$ 0.33	60.96 $\pm$ 9.21
Silymarin	6.28 $\pm$ 2.81	272.5 $\pm$ 32.8	17.32 $\pm$ 0.92	82.52 $\pm$ 8.64*
Acetylsalicylic acid	13.65 $\pm$ 0.91*	324.5 $\pm$ 16.3*	216.67 $\pm$ 42.16*	74.27 $\pm$ 13.68*
Acetylsalicylic acid and silymarin	9.38 $\pm$ 2.53*	300.9 $\pm$ 8.6*	47.56 $\pm$ 4.22*	67.63 $\pm$ 4.56
Naproxen	12.92 $\pm$ 2.91*	317.5 $\pm$ 43.0*	72.42 $\pm$ 8.11*	49.73 $\pm$ 7.40*
Naproxen and Silymarin	10.70 $\pm$ 2.79*	288.6 $\pm$ 14.4	41.36 $\pm$ 6.50*	52.83 $\pm$ 19.21

\* $P < 0.05$  as compared to the control group.

### MATERIALS AND METHODS

Male adult Wistar rats (250-300g) were obtained from the Central Experimental Animal House of the Silesian School of Medicine. Animals were divided into six groups of 10 animals each. Rats were kept in single cages and received standard pellet chow "Murigran" and tap water *ad libitum*. Acetylsalicylic acid ("Polfa", Poland) or naproxen ("Krka", Slovenia) were given orally in daily doses of 150 mg/kg b.w. and 125 mg/kg b.w., respectively. Drugs were given for a 6 week period. In the last 3 weeks of treatment, some animals received silymarin (per os, in a daily dose of 17.5 mg/kg b.w.). When the experiment was over, blood was obtained from the heart and the animals were killed. The following indices were measured in blood serum: bilirubin and total protein levels, activity of alanine aminotransferase, and  $\gamma$ -glutamyl transpeptidase. The methods described by Kokot<sup>11</sup> were used. The results were analysed statistically with Student-t test.

### RESULTS AND DISCUSSION

The obtained results are summarized in Table I. It was found that silymarin alone had no effect on indices of hepatic function except for an increase in total protein levels. Treatment with acetylsalicylic acid led to an elevation of the bilirubin level and the activity of alanine aminotransferase and  $\gamma$ -glutamyl transpeptidase. The total protein level was also increased. Concomitant

treatment with silymarin resulted in some decrease in the elevated indices as compared to rats receiving acetylsalicylic acid only, but results were still higher than those of the control group of animals. The trend of changes seen in rats treated with naproxen with or without silymarin was similar to those found in rats receiving acetylsalicylic acid. Naproxen was found to be less hepatotoxic than acetylsalicylic acid. It is of interest that naproxen led to some decrease in the serum total protein level.

The obtained results indicate a new but tentative application of silymarin, i.e. protection against hepatic damage induced by non-steroidal anti-inflammatory drugs. However, the use of silymarin for this clinical application needs further studies, including histopathological and histochemical evaluation of the liver.

The mechanism of the hepatoprotective activity of silymarin is known only partially. The drug has a capacity to stabilize cellular membranes and act as an antioxidant. Mourelle and Favari<sup>12</sup> reported that silymarin normalized acetylsalicylic acid metabolism in cirrhotic rats but had no effect on salicylate metabolism and disposition in rats with undamaged livers. It is possible that the application of non-steroidal drugs impairs hepatic function and the drugs' detoxication, thus further treatment with anti-inflammatory agents leads to more significant hepatotoxicity due to a longer half-life of the drug in serum. Silymarin may inhibit this self-accelerating mechanism of hepatotoxicity. Possibly, the binding of silymarin to microsome membranes makes them less

susceptible to the deleterious action of the drugs. Mitochondrial enzymes are responsible for drug metabolism. Other mechanisms of silymarin action may also be taken into consideration. Silymarin was found to induce mitochondrial free calcium release which may protect against mitochondrial damage.<sup>13</sup> The drug was also found to affect some connective tissue degrading enzymes.<sup>14</sup>

Although the precise mechanism of hepatoprotective activity of silymarin remains unknown, the application of silymarin may be of benefit in patients undergoing long-term treatment with non-steroidal anti-inflammatory drugs.

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