

Research Article

Protective Effects of Silymarin on Acetaminophen-Induced Toxic Hepatitis

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Abstract

Objectives: Acetaminophen can cause liver damage that can result with death when it is taken as high doses. In our study we proposed to examine the silymarin effect on acetaminophen induced hepatotoxicity.

Methods: In this study, 40 wistar albino rats are randomly divided into 4 groups. 1 ml 0.9% serum physiologic was injected to intraperitoneal (IP) area in control group (group 1). 100 mg/kg silymarin was also injected to IP area in second group (group 2). 1250 mg/kg acetaminophen was injected to IP area in toxic group (group 3). And after 4 hours 1250 mg/kg IP injection of acetaminophen to treatment group (group 4) 100 mg/kg of silymarin was administered to this group. All rats were sacrificed after 24 hours.

Results: In treatment group, acetaminophen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, glutamyltransferase, total bilirubin, direct bilirubin levels which are found increased in toxic group, are found as decreased and statistically significant. The malondialdehyde, a lipid peroxidation product, levels are high in toxic group and low in treatment group and it was statistically significant. There was no significant difference between groups, liver histopathological features.

Conclusion: With this findings it can be assumed that silymarin has hepatoprotective effect.

Keywords: Acetaminophen, silymarin, toxic hepatitis

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Acetaminophen, which is understood to be the active metabolite of phenacetin and acetanilide, which Von Mering first used in 1893, was widely used after World War II.^[1-3]

The average dose for antipyretic and analgesic effects is 1 gram per day. It is administered at a dose of 0.5-1 g 3-4 times a day at most. Acetaminophen toxicity particularly leads to impairment of liver and kidney function. Toxicity occurs at a single dose of 10 grams or doses above 150 mg/kg on average.

Acetaminophen metabolism occurs mainly in the liver and to a lesser extent in the kidneys and intestines. There are three main mechanisms in liver metabolism: glucuronide conjugation, sulfate conjugation, and cytochrome p450 microsomal oxidation.^[2-6] The primary metabolism takes place by glucuronide conjugation with uridine diphosphate glucuronidyl transferase. It is a toxic metabolite of N-acetyl-P-Benzoquinone Imine (NAPQI), which is formed due to the microsomal oxidation of the drug due to cyp450. This toxic

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product binds glutathione in the tissue to non-toxic mercapturic acid and cysteine and is excreted in the urine. In the therapeutic doses of the drug, toxic effects are prevented by glutathione (GSH), which is stored in the body tissue.^[3,5,7]

The two most important mechanisms in acetaminophen-induced toxic hepatitis are the covalent binding of NAPQI to hepatocytes and the oxidative stress and lipid peroxidation caused by it.^[8] Oxidative stress; is the disruption of the balance between free radicals formed due to metabolism in tissues and antioxidant defense and repair systems. When free radicals, in other words, reactive oxygen species (ROS), increase in the cell, the cell's DNA, carbohydrate, protein, and lipid structure deteriorates. ROS increase and lipid peroxidation play an essential role in the etiopathogenesis of many diseases. One of the most important consequences of cell damage with ROS is lipid peroxidation.^[12]

Lipid peroxidation, which occurs at a low rate in normal metabolism, increases oxidative stress and excess oxidants. As a result of lipid peroxidation, malondialdehyde (MDA), aldehyde and hydrocarbon gases rise in the environment. The level of MDA in tissue serum and body fluids of these waste products, mutagenic, genotoxic, and carcinogenic, is used as an indicator of lipid peroxidation.^[13]

Silybum marianum is a plant known in Mediterranean and European countries since ancient times. In the early years of Christianity, it was called "Marian Thistle," after the Virgin Mary. Silymarin consists of 70-80% flavonoid and 20-30% polyphenic structures. Silymarin contains the most efficient and effective silybin. In adults, the capsule is used two or three times a day, 100-300 mg/kg. When taken orally, it reaches the highest serum level in 4-6 hours. Since it is not soluble in water, most of it is excreted through bile, and a small part is excreted in the urine.^[14,15]

It is reported that Silymarin has a positive impact on diseases related to substances that are toxic to the liver, such as acetaminophen and carbon tetrachloride. Silymarin is thought to be protective on the liver utilizing adjusting the amount of glutathione in the cell, activating RNA polymerase by stimulating liver cell regeneration, stabilizing the cell membrane of toxic substances to the liver, preventing toxic substances from being taken into hepatocytes, scavenging free oxygen radicals, and preventing lipid peroxidation.^[16-22] In the present research, we evaluated the effect of silymarin on acetaminophen induced hepatotoxicity in the rats.

Methods

This study was carried out in Trakya University Faculty of Medicine, at the Departments of Internal Diseases, Gastroenterology, Physiology, Pathology, Central Biochemistry and Experimental Animals Laboratory in 2014.

40 male Wistar Albino rats with an average weight of 200±20 gr were used in the study. The subjects were randomly divided into four groups of 10 rats with similar average weights. It was kept under laboratory conditions at a standard temperature of 22±1°C and 12 hours of light/dark light period. Tap water and rat food were used for feeding.

- Group 1 (n=10): It was accepted as the healthy control group. 1 ml of saline was given by intraperitoneal (IP) injection.
- Group 2 (n=10): It was accepted as the Silymarin group. 1 ml of silymarin dissolved with 100 mg/kg dimethyl sulfoxide was given IP.
- Group 3 (n=10): Considered as toxic group. 1250 mg/kg acetaminophen was dissolved in saline and 1 ml IP. was given.
- Group 4 (n=10): It was accepted as the treatment group. Four hours after applying 1250 mg/kg acetaminophen, 100 mg/kg silymarin was dissolved with dimethyl sulfoxide, and 1 ml was given IP.
- 24 hours after acetaminophen administration, rats in 4 groups were anesthetized with 50 mg/kg intramuscular (IM) ketamine and 10 mg/kg rompun. They were sacrificed after drawing their intracardiac blood. Then, livers were removed by opening the abdominal cavities. Tissue samples were taken on blotting papers on ice dishes, divided into three parts with a scalpel. A part of the liver was placed in a 10% formalin solution to be examined under light microscopy; the other liver parts were washed with saline, dried with blotter paper, wrapped in previously coded aluminum foils, and placed in sealed bags. MDA was stored at -80°C until GSH levels were studied.
- Blood samples were centrifuged at +4 C at 3000 g for 10 minutes in a cooled centrifuge, and serum samples were taken into Eppendorf tubes and stored at -80 C.
- ALT, AST, ALP, GGT, T.BİL, and D.BİL were studied from blood samples.
- Spectrophotometric methods examined MDA and GSH levels in the tissue.
- The liver, which was cut sagittally and fixed in 10% formalin to be examined under a light microscope, was embedded in paraffin blocks. After this process, 4 micrometer thick sections were taken, stained with hematoxylin-eosin (HE) dye, and evaluated with a light microscope.

Statistical Analyses

Results were expressed as mean±standard deviation. Whether the parameters fit the normal distribution or not was evaluated with the Kruskal-Wallis test. Differen-

es between all groups were evaluated with ANOVA test and LSD correction. Differences between pairs with statistical significance were determined by Student's t-test for parameters with normal distribution, and by a non-parametric test (Mann-Whitney U test) for parameters that were not normally distributed. A value of $p < 0.05$ was accepted as the cut-off value of statistical significance. SPSS 17.0 (License No: 10240642) version was used in the evaluation of the study.

Results

In the study investigating the therapeutic effects of silymarin in toxic hepatitis induced by acetaminophen, a total of 4 groups were formed: the healthy control group given saline, the group given only silymarin, the toxic group given acetaminophen, and the treatment group given silymarin 4 hours after institution of acetaminophen. Results were compared between groups.

The mean values of biochemical parameters were as follows:

Mean ALT value: 715 ± 175.2 U/L ($p < 0.001$) in acetaminophen induced toxic hepatitis group, mean ALT value: 62.4 ± 15.3 U/L in control group, mean AST value: 1413 ± 414.4 U in toxic hepatitis group/L ($p < 0.001$), mean AST value: 107.2 ± 20.5 U/L in control group, mean ALP value: 321 ± 53.5 U/L ($p < 0.001$) in toxic hepatitis group, mean ALP value: $180, 7 \pm 35$ U/L in control group, mean GGT value: 3 ± 3.71 mg/dl in toxic hepatitis group, mean GGT value: 1.6 ± 0.9666 mg/dl in the control group, mean T.BIL value: 0.42 ± 0.09 mg/dl in toxic hepatitis group ($p < 0.001$), mean T.BIL value: 0.13 ± 0.0675 mg/dl in the control group, mean D.BIL value: 0.11 ± 0.031 mg/dl ($p < 0.05$) in the toxic hepatitis group, and 0.05 ± 0.053 mg/dl. in the control group. The mean MDA value showing oxidant activity in the liver was 1.797 ± 0.148 nmol/g ($p < 0.001$) in the toxic hepatitis group, and the mean MDA value was 1.094 ± 0.292 nmol/g in the control group. The mean GSH value showing antioxidant activity in the liver was 6.39 ± 1.55 μ mol/gr in the toxic hepatitis group, and the mean GSH value in the control group was 5.57 ± 0.472 μ mol/gr. The difference was not statistically significant (Table 1).

Mean ALT value in silymarin-treated group was 153 ± 60.51 U/L ($p < 0.001$) whereas mean ALT value was 715 ± 175.2 U/L in toxic hepatitis group; mean AST value in the treatment group was 266 ± 117.012 U/L ($p < 0.001$), mean AST value in the toxic hepatitis group was 1413 ± 414.4 U/L; mean ALP value was 192.7 ± 30.631 U/L ($p < 0.001$) in the treatment group, mean ALP value in the toxic hepatitis group was 321 ± 53.5 U/L; mean GGT value in the treatment group was 1.7 ± 0.823 mg/dl, mean GGT value was 3 ± 3.71 mg/dl in the toxic hepatitis group; mean T.BIL value was 0.16 ± 0.69

mg/dl ($p < 0.001$) in the treatment group, mean T.BIL value in toxic hepatitis group was 0.42 ± 0.09 mg/dl; mean D.BIL value was 0.04 ± 0.052 mg/dl ($p < 0.05$) in the treatment group, while it was 0.11 ± 0.032 mg/dl in the toxic hepatitis group. The mean MDA value was found to be 1.548 ± 0.098 nmol/gr ($p < 0.05$) in the silymarin treatment group, and the mean MDA value in the toxic hepatitis group was found to be lower than 1.797 ± 0.148 nmol/gr, which was statistically significant. The mean GSH value was found to be 6.653 ± 0.7046 μ mol/gr in the silymarin treatment group, and the mean GSH value in the toxic hepatitis group was found to be higher than 6.39 ± 1.55 μ mol/gr, which was not statistically significant (Table 2).

The mean ALT value in the silymarin-treated group was 153 ± 60.51 U/L ($p < 0.05$), in the control group, the mean

Table 1. Comparison of blood biochemical parameters, liver tissue malondialdehyde and glutathione levels between the control group and the toxic hepatitis group

	Control group mean values	Toxic hepatitis group mean values	p
ALT	62.4 ± 15.4	715 ± 175.2	<0.001*
AST	107.2 ± 20.5	1413 ± 414.5	<0.001*
ALP	180.7 ± 35	321.7 ± 53.6	<0.001*
GGT	1.6 ± 1	3 ± 3.8	0.240
T.BiL	0.13 ± 0.06	0.420 ± 0.09	<0.001*
D.BiL	0.05 ± 0.052	0.11 ± 0.031	<0.001*
MDA	1.094 ± 0.29	1.797 ± 0.148	<0.001*
GSH	5.57 ± 0.472	6.39 ± 1.55	0.103

*: A value of $p < 0.05$ was accepted as the cut-off value of statistical significance. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase; T.BiL: Total Bilirubin; D.BiL: Direct Bilirubin; MDA: Malondialdehyde; GSH: Glutathione.

Table 2. Comparison of blood biochemical parameters, liver tissue malondialdehyde and glutathione levels between the toxic hepatitis group and the treatment group

	Toxic hepatitis group mean values	Silymarin-treated group mean values	p
ALT	715 ± 175.2	153.9 ± 60.51	<0.001*
AST	1413 ± 414.5	266.8 ± 117	<0.001*
ALP	321.7 ± 53.6	192.7 ± 30.6	<0.001*
GGT	3 ± 3.8	1.7 ± 0.8	0.626
T.BiL	0.420 ± 0.09	0.16 ± 0.69	<0.001*
D.BiL	0.11 ± 0.031	0.04 ± 0.052	<0.05*
MDA	1.797 ± 0.148	1.548 ± 0.098	<0.05*
GSH	6.39 ± 1.55	6.65 ± 0.7	0.634

*: A value of $p < 0.05$ was accepted as the cut-off value of statistical significance. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase; T.BiL: Total Bilirubin; D.BiL: Direct Bilirubin; MDA: Malondialdehyde; GSH: Glutathione.

ALT value was 62.4 ± 15.3 U/L, in the treatment group; the mean AST value was 266 ± 117.012 U/L ($p < 0.05$) in the control group, the mean AST value was 107.2 ± 20.5 U/L in the treatment group; the mean MDA value was found to be 1.548 ± 0.098 nmol/gr ($p < 0.001$) in the treatment group, and the mean MDA value in the control group was higher than 1.094 ± 0.292 nmol/gr, which was statistically significant. No statistical significance was found in the comparison of other parameters between the treatment group and control group with Silymarin (Table 3).

There was no statistically significant difference between the parameters that were compared between the control group that received saline and the group that received only silymarin (Table 4).

Table 3. Comparison of blood biochemical parameters, liver tissue malondialdehyde and glutathione levels between the control group and the treatment group

	Control group mean values	Silymarin-treated group mean values	p
ALT	62.4 ± 15.4	153.9 ± 60.51	<0.05*
AST	107.2 ± 20.5	266.8 ± 117	<0.05*
ALP	180.7 ± 35	192.7 ± 30.6	0.897
GGT	1.6 ± 1	1.7 ± 0.8	0.240
T.BiL	0.13 ± 0.06	0.16 ± 0.69	0.845
D.BiL	0.05 ± 0.052	0.04 ± 0.052	0.999
MDA	1.094 ± 0.29	1.548 ± 0.098	<0.001*
GSH	5.57 ± 0.472	6.65 ± 0.17	0.103

*: A value of $p < 0.05$ was accepted as the cut-off value of statistical significance. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase; T.BiL: Total Bilirubin; D.BiL: Direct Bilirubin; MDA: Malondialdehyde; GSH: Glutathione.

Table 4. Comparison of blood biochemical parameters, liver tissue malondialdehyde and glutathione levels between the control group and the silymarin group

	Control group mean values	Silymarin-treated group mean values	p
ALT	62.4 ± 15.4	74.8 ± 23.5	0.701
AST	107.2 ± 20.5	122.2 ± 12.3	0.337
ALP	180.7 ± 35	208.8 ± 29.2	0.110
GGT	1.6 ± 1	2.6 ± 3.5	0.701
T.BiL	0.13 ± 0.06	0.2 ± 0.094	0.241
D.BiL	0.05 ± 0.052	0.08 ± 0.06	0.842
MDA	1.094 ± 0.29	1.349 ± 0.31	0.107
GSH	5.57 ± 0.472	5.86 ± 1.3	

*: A value of $p < 0.05$ was accepted as the cut-off value of statistical significance. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase; T.BiL: Total Bilirubin; D.BiL: Direct Bilirubin; MDA: Malondialdehyde; GSH: Glutathione.

Discussion

In the present study where we examined the effects of silymarin in the experimental hepatotoxicity model created with acetaminophen, serum ALT, AST, ALP, T. BiL, D. BiL values in the toxic group were found to be statistically significant and higher than the control group. Serum ALT, AST, ALP, T. BiL and D. BiL values were found to be statistically significant and lower in the silymarin treatment group compared to the toxic group.

Selvam et al.^[23] investigated the positive effects of silymarin and another antioxidant, *S. jambos*, on paracetamol hepatotoxicity. It was shown that ALT, AST, ALP, T.BiL, D.BiL levels were significantly increased in rats with hepatotoxicity, and they were significantly improved in the treatment groups. In this study, all rat groups were given silymarin for nine days and paracetamol on the last day. Histopathologically, they found that paracetamol toxicity approached normal levels in the group treated with silymarin.

Murali et al.^[24] investigated the effects of smilax zeylanica on paracetamol-induced liver toxicity; in the toxic group, ALT, AST, T.BiL, ALP levels were statistically significantly increased in the toxic group compared to the control group, the values in the treatment group were close to the control group and the toxic group. They found that the values are statistically significant.

In our study, no significant difference was found between the groups in terms of GGT levels measured in serum. Lebda et al.^[25] examined the effects of ginger on paracetamol-induced chronic hepatotoxicity and found that serum GGT levels increased in the treatment group, and there was no significant difference between the other groups. Also, in the literature review, there were not many studies on GGT levels in acetaminophen hepatotoxicity.

The levels of MDA, which is an indicator of lipid peroxidation in liver toxicity, increased in the toxic group compared to the control group and decreased in the treatment group compared to the toxic group, and the differences were found to be statistically significant and consistent with the current literature.

GSH levels used as an antioxidant indicator in liver toxicity were similar in all four groups, and no difference was found between the groups. Mirochnitchenko et al.^[26] found in their acetaminophen hepatotoxicity study that liver GSH levels decreased in the toxic group at the 1st hour and returned to normal levels at the 8th hour. Similarly, Meotti et al.^[27] found that hepatic GSH decreased 4 hours after giving APAP and returned to normal after 24 hours and explained this situation with antioxidant defense. In our study, desired GSH levels may not have

been reached due to the sacrifice of the subjects at the 24th hour.

In experimental studies, it is stated that in acetaminophen toxicity, histopathologically necrosis, inflammation, and balloon degeneration are observed in the liver. In our study, no significant histopathological findings were found in any group, including toxic group.

Selvam et al.^[23] developed paracetamol toxicity at a high dose (2500 mg/kg) after eight days of silymarin treatment. It was observed that diffuse hemorrhage and necrosis were seen histopathologically in the toxic group, and it was customary in those who received silymarin treatment.

Shito et al.^[28] in their study investigating the time of fulminant hepatitis necrosis with galactosamine in rats, found that the necrosis they developed occurred precisely at 48 hours.

In Eskişehir Osmangazi University, Orhan et al.^[29] found that necrosis occurred at 72nd hour in all rats in a preliminary study investigating acetaminophen-induced necrosis and the development time of acetaminophen-induced necrosis effectiveness of flumazenil. As stated above, the absence of necrosis at the 24th hour with a single dose of 1250 mg/kg acetaminophen was observed to be related to the protocol of the present study.

Conclusion

As a result, it was determined that acetaminophen at a dose of 1250 mg/kg caused an increase in liver transaminases and tissue MDA levels in rats. Silymarin provided a significant improvement in serum transaminases and decreased the level of MDA in liver tissue. With these findings, it can be thought that silymarin has a hepatoprotective effect.

Disclosures

Ethics Committee Approval: Trakya University Animal Experiments Local Ethics Committee Decision. Date: 05.04.2013 Number: 2013.03.06.

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