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Effect of Methoxsalen and an Infusion of *Silybum marianum* on Enzymes Relevant to Liver Function

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Abstract

The effect of an infusion of *Silybum marianum* on some enzymes relevant to liver function was investigated in mice. Methoxsalen was administered in doses of 2.5 and 5.0 mg/kg (i.p.) 4 h prior to decapitation. The infusion of *Silybum marianum* was administered (p.o.) 24 and 2 h before methoxsalen, while the third dose was given together with methoxsalen. The animals were sacrificed 4 h later. The results showed that the content of glutathione, cytochrome P450 and transaminase in animal tissues suggests the hepatoprotective action of *Silybum marianum* infusion.

Keywords: *Silybum marianum*, methoxsalen, hepatoprotection.

Introduction

There is a common belief that plant-originated drugs are harmless/nontoxic, which cannot be assumed as true. The opinion that herbal products are not toxic was formed when it still was not possible to ascertain harmful effects (carcinogenicity, mutagenicity, hepatotoxicity, etc.) that are manifested only after a prolonged consumption of herbal preparations. Unfortunately, there have only been a small number of preclinical and clinical studies of herbal preparations and teas that are so commonly in use.

Silybum marianum (L.) is a biennial herbaceous weed plant growing wild on the uncultivated lands of southern Europe, South and North America. The most important pharmacologically active substances in its above-ground parts are histamine and tyramine. The plant's fruits contain flavonoids, fatty oils, various sugars, essential oils, and tyramine. *Silybum marianum* contains silymarin, which is known as a hepatoprotective flavonolignan (Strubelt et al., 1980). The major constituent of silymarin is a silybin, which accumulates liver glutathione, and thereby increases the resistance of hepatocyte membranes (Valenzuela et al., 1989). In folk medicine, it is used in the form of teas in cases of asthma, headache, hypotension, as well as a bitter appetizing agent, for mitigation of pains related to liver function, etc. Preliminary experiments showed some hepatoprotective action of the plant extract with animals intoxicated with carbon tetrachloride (List & Horhammer 1979; Barzaghi, 1990; Tucakov, 1990).

In this work, we investigated the individual effects of an infusion of *Silybum marianum* and two doses of methoxsalen (inhibitor of cytochrome P450 activity) on the content of some enzymes related to liver function, as well as the interaction of methoxsalen and the infusion by measuring the same parameters in mice.

Materials and methods

Experimental animals and groups

Experiments were carried out on white laboratory mice of both sexes, HAN : NMR breed, body weight 20–30 g. The animals had free access to food and water and were maintained at 20–22°C with a 12 h light period. The animals were divided into the following groups with 6 animals in each group:

C_0, control, received only physiological solution;
C_1, treated with the infusion, 28, 6, and 4 h before decapitation;
C_2, treated with methoxsalen, 2.5 mg/kg, 4 h before decapitation;
C_3, treated with methoxsalen, 5.0 mg/kg, 4 h before decapitation;

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E_1, treated with the infusion 24 and 2 h before administering 2.5 mg/kg of methoxsalen, the third dose of infusion being given together with methoxsalen;
E_2, treated with the infusion 24 and 2 h before administering 5.0 mg/kg of methoxsalen, the third dose of infusion being given together with methoxsalen.

Infusion

Boiling water (100 ml) was poured over dry and ground fruits of *Silybum marianum* (5 g) and left to macerate for 30 min. The cooled and filtered infusion was given to mice p.o. (10 ml/kg). Infusion was prepared fresh every day.

Drugs: Methoxsalen in the form of pure substance dissolved in olive oil was given i.p. in a dose of 2.5 mg/kg (group C_2) or 5.0 mg/kg (group C_3).

Biochemical methods

The contents of the relevant enzymes in the serum (transaminase) and liver homogenate (glutathione, cytochrome P450 and total protein) were assayed. Cytochrome P450 was determined after Matsubara et al. (1976) and glutathione after Kapetanović and Mieyal (1979). Contents of transaminases (SGOT and SGPT) were measured spectrophotometrically using kits from Merck S.A. Brazil.

Biological material

After killing the mice, decapitation blood was collected and, after preparation, the liver was weighed and homogenized in ice-cold Tris–HCl sucrose buffer, pH 7.4.

Statistical analysis

Statistical evaluation of the results was done by the Student unpaired *t*-test (Tallarida & Murray, 1986).

Results and discussion

Levels of SGOT are presented in Table 1. As can be seen, all treatments caused an increase in SGOT, and statistical significance is observed in the group treated with infusion alone, as well as in the groups treated with both doses of methoxsalen. The combination of the infusion and methoxsalen showed significant differences with respect to the controls. A certain decrease in the SGPT value in the serum can be observed, but this decrease is not statistically significant. When we compared groups C_2 and E_1, the data showed that the infusion significantly prevents an increase of SGOT caused by methoxsalen.

The content of glutathione in liver homogenate is presented in Figure 1. All treatments yielded a statistically significant decrease of glutathione content in the liver homogenate in relation to the control. The infusion administered to mice according to the above scheme did not sustain the glutathione level in the liver homogenate; moreover, the infusion itself caused a significant drop of this parameter. However, the group treated with a combination of infusion and methoxsalen (E_1) has a significantly higher content of glutathione in relation to the group treated only with methoxsalen (C_2).

Table 1. The influence of methoxsalen and *Silybum marianum* infusion on the content of SGOT in mouse serum. C_0-control, C_1-infusion, C_2-methoxsalen 2.5 mg/kg, C_3-metoxsalen 5 mg/kg, E_1-methoxsalen 2.5 mg/kg and infusion, E_2-methoxsalen 5 mg/kg and infusion.

Group	SGOT (U/L)
C_0	145.2 ± 8.7
C_1	165.7 ± 6.5*
C_2	159.3 ± 2.3*,**
C_3	161.2 ± 4.2*
E_1	150.6 ± 5.3
E_2	151.3 ± 3.7

n = 6/group.
* $p < 0.05$ in relation to control.
** $p < 0.05$ in relation to E_1.

The content of cytochrome P450 in the liver homogenate is presented in Figure 2. As can be seen, certain irregularities were observed with this parameter. Namely, the lower dose of methoxsalen (2.5 mg/kg) caused a statistically significant decrease in cytochrome P450 content, whereas the decrease with the dose of 5.0 mg/kg did not attain statistical significance. The infusion application according to the above scheme sustained a significant decrease of the cytochrome value in the animals that received the lower dose of methoxsalen (2.5 mg/kg), whereas in the group receiving the higher methoxsalen dose (5.0 mg/kg), it apparently potentiated the methoxsalen effect in lowering the cytochrome P450 level in the liver homogenate.

Fouin-Fortunet et al. (1986) reported that methoxsalen shows a hepatotoxic action and that it is suitable for laboratory tests of hepatotoxicity and hepatoprotective effects on experimental animals. Using high doses (exceeding 25 mg/kg), they could not prove the existence of the dose dependence. At methoxsalen doses of 2.5 and 5.0 mg/kg, no dose dependence could be established either in this work or in our previous study (Jakovljević et al., in press).

The existence of a hepatoprotective effect of the *Silybum marianum* infusion could be postulated on the basis of measuring the contents of transaminases. Namely, the injury of hepatocytes, similar to that caused by carbon tetrachloride, observed with both methoxsalen doses, caused a significant increase of SGOT in the liver homogenate, whereas treatment of the animals with the infusion prevented a statistically significant increase in the SGOT level. No significant changes in the SGPT values were observed in any case of the pretreatment scheme.

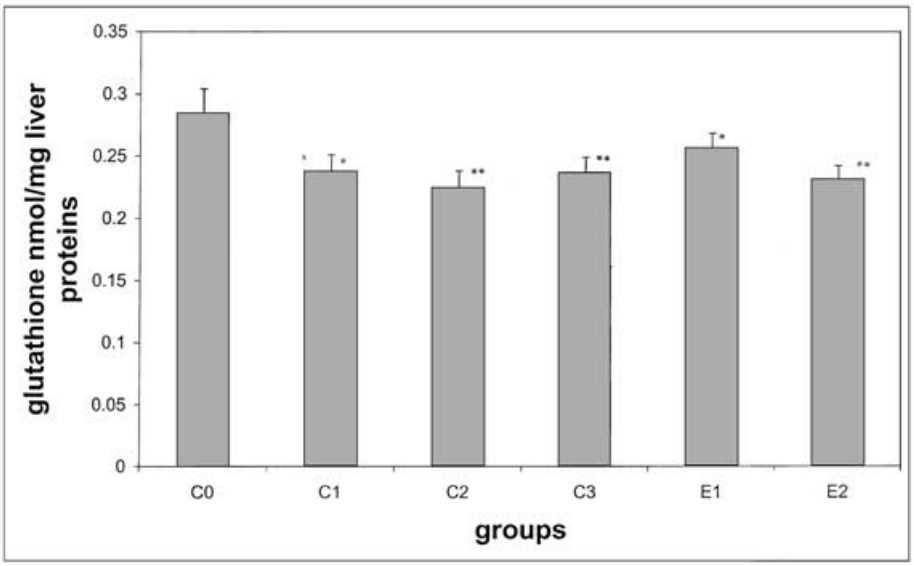

Figure 1. The influence of methoxsalen and *Silybum marianum* infusion on the content of gluthatione in the liver homogenate of mice. C_0-control, C_1-infusion, C_2-methoxsalen 2.5 mg/kg, C_3-methoxsalen 5.0 mg/kg, E_1-methoxsalen 2.5 mg/kg and infusion, E_2-methoxsalen 5.0 mg/kg and infusion. n = 6/group. *$p < 0.05$, **$p < 0.01$ in relation to control, $^{x}p < 0.05$ in relation to E_1.

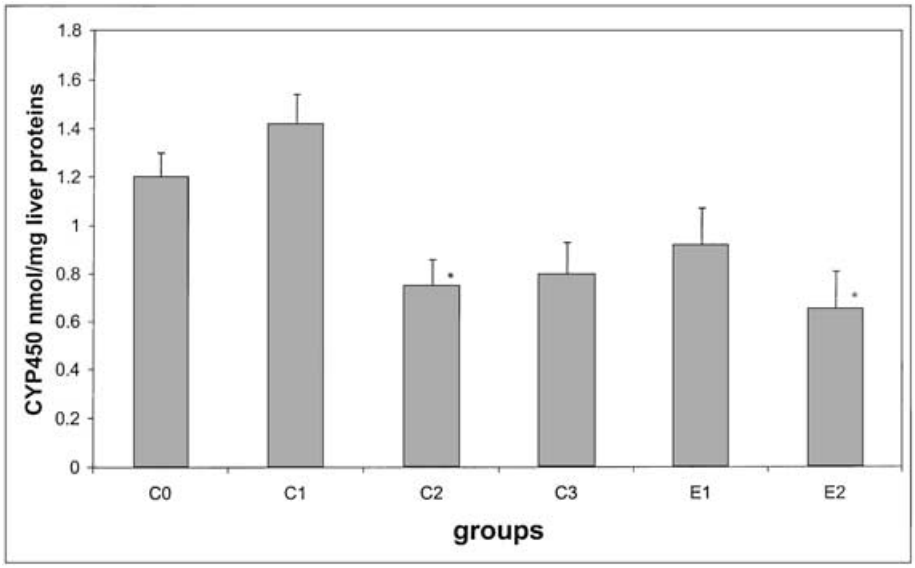

Figure 2. The influence of methoxsalen and *Silybum marianum* infusion on the content of cytochrome P450 in the liver homogenate of mice. C_0-control, C_1-infusion, C_2-methoxsalen 2.5 mg/kg, C_3-methoxsalen 5.0 mg/kg, E_1-methoxsalen 2.5 mg/kg and infusion, E_2-methoxsalen 5.0 mg/kg and infusion. n = 6/group, *p < 0.05 in relation to control.

On the basis of the results of determining content of glutathione, transaminases, and cytochrome P450 in mice, it can be concluded that there is a hepatoprotective effect of the infusion of *Silybum marianum* when methoxsalen is used as a hepatotoxic agent.

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