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Effect of *Viscum album* on acute hepatic damage caused by carbon tetrachloride in rats

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Aim: To investigate the effect of *Viscum album*, a plant used for the treatment of hepatocellular carcinoma that has immune-modulating properties, on acute hepatic injury in rats.

Materials and methods: Hepatotoxicity was induced by CCl_4 orally (0.28 mL/kg). Rats received either *Viscum album* at 1 of 2 dose levels (0.1 or 0.2 mL/kg) once weekly subcutaneously alone or with silymarin (25 mg/kg, orally), or silymarin (25 mg/kg) once daily orally for 1 month, starting at the time of administration of CCl_4. Liver damage was assessed by determining liver serum enzyme activities and by hepatic histopathology.

Results: *Viscum album* administration decreased the increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and also prevented the development of hepatic necrosis caused by CCl_4. The effect of *Viscum album* was dose-dependent. *Viscum album* administered at 0.1 or 0.2 mL/kg caused significant reduction in the elevated plasma ALT by 51.2% and 65.6%, AST by 52.6% and 61.1%, and ALP by 27.7% and 57.6%, respectively. In comparison, the elevated serum ALT, AST, and ALP levels decreased to 48.9%, 51.8%, and 30.8% of the controls, respectively, with 25 mg/kg of silymarin. *Viscum album* (0.2 mL/kg) administered together with silymarin resulted in 73.1%, 67.6%, and 65.8% decreases in serum ALT, AST, and ALP levels, respectively. Histopathologic examination of the livers of rats treated with CCl_4 and administered *Viscum album* at 0.2 mL/kg showed marked restoration of the normal architecture of the liver tissue with minimal fibrosis.

Conclusion: Results of the present study indicate that the administration of *Viscum album* in a model of liver injury induced by CCl_4 in rats results in less liver damage.

Key words: *Viscum album*, hepatic injury, rat, CCl_4

Introduction

Currently, the standard therapy for patients with hepatitis C virus infection involves the combined administration of interferon and ribavirin (1,2). With the use of interferon and ribavirin, sustained response is obtained in approximately 55% of patients (3,4). Despite the fact that this combination therapy can eliminate the virus and prevent the progression of the disease, frequent side effects and relapses preclude the use of this form of therapy in a substantial proportion of patients (4,5). Thus, it is clear that there is still a need for the identification of compounds that are capable of modulating chronic inflammation in the liver of such patients because hepatic inflammation appears to be the key pathological substrate that drives fibrogenesis (6,7).

Apart from interferon-ribavirin therapy, silymarin, a standardized extract of milk thistle (*Silybum marianum*), is widely prescribed for chronic liver disease (8). Silymarin possesses antioxidant properties attributable to its flavonoid components, silibinin, silicristin, and silidianin (9). In animal models of hepatic injury, the herb has been shown to ameliorate liver injury and to

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exert antifibrotic effects (10-13). Studies in patients with chronic hepatitis C and alcoholic liver disease have shown that silymarin increases superoxide dismutase activity of lymphocytes and erythrocytes (14), and increases glutathione and decreases lipid peroxidation in peripheral blood cells (15,16). Silymarin, however, does not appear to affect viral load or liver histology in hepatitis B or C (17).

Recently, patients with chronic hepatitis C treated with *Viscum album* preparations had a significant improvement of both AST and ALT (18). *Viscum album* preparations consist of aqueous extracts of *Viscum album* L., the European mistletoe (family Loranthaceae). The latter is a semiparasitic plant that is normally found growing on a variety of trees in temperate regions worldwide. European mistletoe preparations are aqueous extracts obtained from the whole plant. They have cytotoxic and immunomodulatory properties and are among the most widely used unconventional cancer therapies in Germany and Central Europe (19,20). In Germany, mistletoe extract is listed in certain directories as a commercially available drug for cancer treatment (21).

Since there is a need to explore alternative therapies for chronic liver disease, the present study aimed to investigate the effect of *Viscum album* on the development of experimental liver damage induced by the administration of the hepatotoxin CCl_4 in rats. The effect of *Viscum album* was evaluated both on biochemical markers as well as by histological techniques and compared with that of silymarin, in view of the wide use of this plant extract as a hepatoprotective agent in patients with chronic hepatitis C.

Materials and methods

Animals

Adult Sprague-Dawley rats of either sex, weighing 120-130 g, were used throughout the experiments and fed with standard laboratory chow and water ad libitum. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals.

Drugs and chemicals

Carbon tetrachloride (BDH Chemicals, England), *Viscum album* (viscum fraxini-2, ATOS Pharma Co., Cairo), and silymarin (Sedico Pharmaceutical Co., Cairo) were used in the experiments. The mistletoe preparation for the study was an aqueous injectable solution. It contained 1 mL of viscum fraxini in dilution stage 2 (15 mg extract of 20 mg mistletoe herb from ash tree, diluted in Na_2HPO_4, ascorbic acid, and water), which is equivalent to 10,000 ng/mL injection ampoules. Further dilution with distilled water was done to obtain the necessary doses. The doses employed were based upon the human dose after conversion to rat doses according to Paget and Barnes' (22) conversion tables.

The carbon tetrachloride model of hepatic damage

The rats were divided into 6 equal groups of 6 rats each. Groups 1-5 received CCl_4 in olive oil (1:1, vol/vol) orally at a dose of 2.8 mL/kg. Rats were administered half the initial dose of CCl_4 (0.14 mL/kg) once weekly after the first administration of CCl_4 so as to maintain hepatic damage. Starting on the first day of CCl_4 administration, rats were treated with saline (CCl_4 control, or group 1), *Viscum album* (at 1 of 2 dose levels, 0.1 or 0.2 mL/kg) once weekly subcutaneously (sc) (groups 2 and 3, respectively), silymarin only (25 mg/kg) orally once daily (group 4), or *Viscum album* (0.2 mL/kg, sc) plus silymarin (25 mg/kg, orally) once weekly (group 5) for 30 days. In addition, another group of rats (n = 6) received saline but no CCl_4 daily for 30 days (group 6). Rats had free access to food and drinking water during the study.

Biochemical assessment

At the end of the experiments, blood samples were obtained from the retro-orbital vein plexuses under ether anesthesia. ALT and AST activities in serum were measured according to the Reitman-Frankel colorimetric transaminase procedure (23), whereas colorimetric determination of ALP activity was done according to the method of Belfield and Goldberg (24), using commercially available kits (bioMérieux, France).

Histopathological studies

After the end of the treatment period, rats were killed under ether anesthesia, and livers were excised

and fixed in 10% formalin saline. Sections were prepared and stained with hematoxylin and eosin (H&E) for histological investigation.

Statistical analysis

All results are expressed as means ± SE. Comparison of the values before and after CCl_4 was made by paired Student's t-test. Multiple group comparisons were performed by ANOVA followed by Duncan's test. $P < 0.05$ was considered statistically significant.

Results

Biochemical results

The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in plasma were significantly higher in CCl_4-treated rats than in the saline control group, indicating the severity of hepatic injury and cholestasis caused by CCl_4. Thus, compared with the saline control group, ALT, AST, and ALP were raised by 222%, 173.4%, and 186.5%, respectively (Table). *Viscum album* administered at 0.1 mL/kg significantly reduced the elevated plasma ALT by 51.2%, AST by 52.6%, and ALP by 27.7%. *Viscum album* at the higher dose of 0.2 mL/kg significantly decreased the raised plasma ALT by 65.6%, AST by 61.1%, and ALP by 57.6%. In comparison, the elevated serum ALT, AST, and ALP levels were decreased to 48.9%, 51.8%, and 30.8% of the controls, respectively, with 25 mg/kg of silymarin. *Viscum album* (0.2 mL/kg) combined with silymarin resulted in 73.1%, 67.6%, and 65.8% decreases in serum ALT, AST, and ALP levels, respectively (Table).

Histopathological results

The livers of saline control rats revealed the characteristic hepatic architecture (Figure 1). The liver of rats subjected to CCl_4 showed loss of liver tissue architecture, severe dilatation and congestion of blood vessels (either central veins or portal tract vessels), marked lymphocytic infiltration, and fibrosis extending between the portal areas (Figure 2). Sections of liver tissue from rats treated with *Viscum album* at 0.1 mL/kg showed slight distortion of the normal architecture of liver tissue with marked dilatation and congestion of the portal veins in portal areas (Figure 3). Sections of liver tissue from rats treated with *Viscum album* at 0.2 mL/kg showed preserved liver architecture with marked dilatation of blood sinusoids, denoting edema with congestion and dilatation of blood vessels (Figure 4).

Discussion

In the present study, acute hepatic injury was induced by injection of CCl_4 in rats, leading to high levels of serum aminotransferases and considerable perivenular necrosis. Hepatic injury was reduced by once weekly administration of *Viscum album* with a reduction in plasma levels of hepatocellular enzymes

Table. Effect of *Viscum album*, silymarin, or *Viscum album* plus silymarin on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in CCl_4-treated rats.

Treatment	ALT (U/L)	AST (U/L)	ALP (IU/L)
Saline control	71.0 ± 3.1	80.4 ± 2.5	50.9 ± 1.9
CCl_4 control	228.6 ± 10.0	219.8 ± 3.7	146.1 ± 6.0
CCl_4 + viscum album 0.1 mL/kg	111.5 ± 3.5*	104.1 ± 3.4*	105.7 ± 4.1*
CCl_4 + viscum album 0.2 mL/kg	78.7 ± 3.1^{*+}	85.5 ± 5.2^{*+}	62.0 ± 5.2^{*+}
CCl_4 + silymarin 25 mg/kg	116.8 ± 6.7*	106.0 ± 8.1*	101.0 ± 7.1*
CCl_4 + silymarin + viscum album 0.2 mL/kg	61.6 ± 3.4^{*+}	71.2 ± 2.5^{*+}	50.0 ± 2.6^{*+}

Results were means ± S.E. Data were analyzed by one-way ANOVA and the means of different groups were compared with Duncan's multiple range test. *: $P < 0.05$ compared with the CCl_4 control group. +: $P < 0.05$ compared with groups treated with *Viscum album* 0.2 mL/kg or silymarin alone.

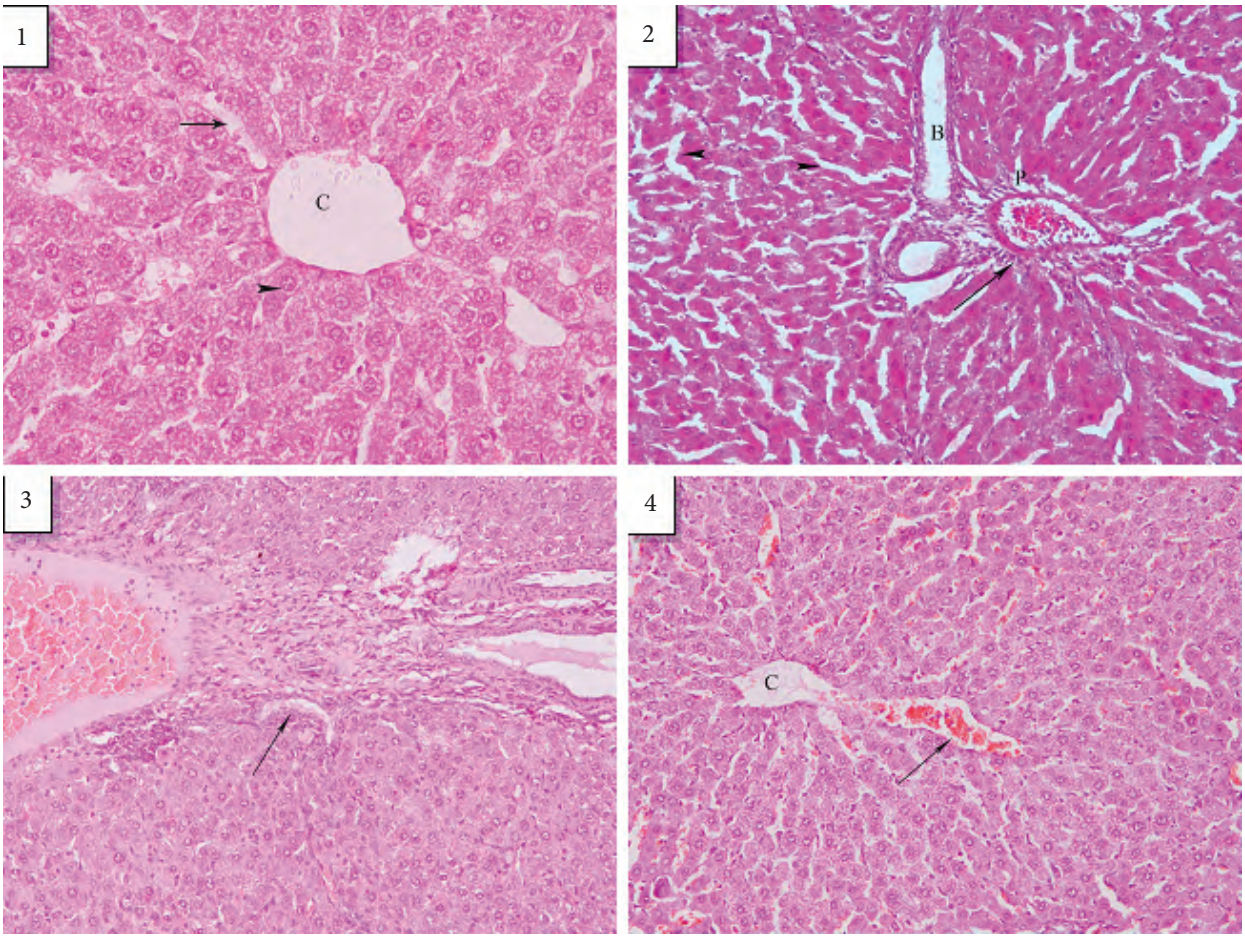

Figure 1-4. (1) A photomicrograph of a section of liver tissue of a control rat showing the central vein and the hepatocytes arranged in cords radiating from the central vein (C) in an anastomosing manner to form a spongework or labyrinth (H&E, ×200).
(2) A photomicrograph of a section of liver tissue of a rat treated with carbon tetrachloride showing distortion of the normal architecture of the liver tissue. Bundles of collagen fibers with fibrocytes (arrow) are surrounding the portal tract components, especially the portal vein (P). These components show dilatation with congestion in the portal vein. The blood sinusoids show dilatation (arrow head), and also the bile duct (B) (H&E, ×200).
(3) A photomicrograph of a section of liver tissue of a rat treated with carbon tetrachloride and 0.1 mL/kg of *Viscum album* showing most of the hepatocytes being normal in appearance and arrangement, with no dilatation of the blood sinusoids. Collagenous fibers with fibrocytes are observed in between the hepatocytes (arrow). Marked dilatation and congestion of blood vessels is seen in the left part of the figure (H&E, ×200).
(4) A photomicrograph of a section of liver tissue of a rat treated with carbon tetrachloride and 0.2 mL/kg of *Viscum album* showing a nearly normal appearance of liver tissue, except for the dilated and congested blood sinusoids (arrow). Both the central vein (C) and the hepatocytes are of normal size and shape (H&E, ×200).

ALT and AST as well as the cell membrane enzyme ALP and with marked improvement in liver morphology. These results pointed to a hepatic protective effect of *Viscum album*. The study also indicated the usefulness of combining both *Viscum album* and silymarin.

Little information is available in the literature on the effect of *Viscum album* on hepatic injury. The plant, however, has been widely investigated for its anticancer properties (19,20). Two recent studies done in patients with chronic hepatitis C, treated with a mistletoe preparation as monotherapy for 1 year,

reported a significant improvement in elevated transaminases (25). The mechanism(s) by which *Viscum album* modulates hepatic inflammation remains, however, unclear. *Viscum album* extracts contain mistletoe lectins, cytotoxic glycoproteins also known as viscumin or agglutinin, which are members of the family of type 2 ribosome-inactivating proteins, and viscotoxin, which is a 46-amino acid peptide that damages cell membranes (26). Other constituents include polysaccharides (galacturonan and arabinogalactan) and alkaloids. *Viscum album* extracts have both immunomodulatory (induces TNF-α and IL-12) and apoptosis-inducing properties, which are likely to be dose-dependant (27). The perioperative administration of *Viscum album* attenuated the immuno-suppressive effects of surgery, increasing the number of NK cells, the T and B cells, complement, and IgA, IgG, and IgM values (28). Studies also suggested that European mistletoe possesses insulin-secreting (29), antihyperglycemic, antioxidant activity (30), and cholinomimetic activities (31) for *Viscum album* L.

Findings in the present study suggest that the use of silymarin with *Viscum album* can have an additive beneficial effect in lessening liver inflammation and necrosis caused by CCl_4. The release of aminotransferases into the plasma was markedly reduced, indicating a reduction in the severity of liver damage by the combination. Aminotransferases are sensitive indicators of liver-cell injury and are released into the blood in increasing amounts whenever the liver cell membrane is damaged (32). Silymarin, a standardized plant extract, has been used for many years as a hepatoprotective agent (8,9). In patients with liver disease, silymarin increased superoxide dismutase activity of lymphocytes and erythrocytes (14), and increased glutathione and decreased lipid peroxidation in peripheral blood cells (15,16). In in vitro models of hepatotoxicity, silymarin decreased lactate dehydrogenase leakage, increased oxygen consumption, reduced the formation of lipid peroxides (malondialdehyde), and reduced the increased Ca^{++} in hepatocytes (33). Silymarin also possesses important antiinflammatory properties, inhibiting the migration of neutrophils into the inflamed site (34), which are likely to be of relevance to its hepatoprotective effects.

Mistletoe preparations are among the most widely used unconventional cancer therapies in many countries (19,20). The results of the present study suggest that mistletoe preparations may be a useful therapeutic intervention for patients with chronic liver disease. Clearly, further studies are required to elucidate the mechanism(s) by which mistletoe preparations exert their hepatoprotective effects seen in the present study.

References

1. Pianko S, McHutchison JG. Treatment of hepatitis C with interferon and ribavirin. J Gastroenterol Hepatol 2000; 15: 581-6.
2. Bacon BR. Managing hepatitis C. Am J Manag Care 2004; 10: S30-S40.
3. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347: 975-82.
4. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358: 958-65.
5. Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology 2002; 36: S237-S244.
6. McCaughan GW, George J. Fibrosis progression in chronic hepatitis C virus infection. Gut 2004; 53: 318-21.
7. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005; 115: 209-18.
8. Wellington K, Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. BioDrugs 2001; 5: 465-89.
9. Flora K, Hahn M, Rosen H, Benner K. Milk thistle (*Silybum marianum*) for the therapy of liver disease. Am J Gastroenterol 1998; 93: 139-43.
10. Muriel P, Mourelle M. Prevention by silymarin of membrane alterations in acute CCl4 liver damage. J Appl Toxicol 1990; 10: 275-79.
11. Muriel P, Garciapina T, Perez-Alvarez V, Mourelle M. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J Appl Toxicol 1992; 12: 439-42.
12. Wu CG, Chamuleau RA, Bosch KS, Frederiks WM. Protective effect of silymarin on rat liver injury induced by ischemia. Virchows Arch B Cell Pathol 1993; 64: 259-63.

13. Jia JD, Bauer M, Cho JJ, Ruehl M, Milani S, Boigk G, Riecken, EO, Schuppan D. Antifibrotic effect of silymarin in rat secondary biliary fibrosis is mediated by downregulation of procollagen alpha1(I) and TIMP-1. J Hepatol 2001; 35: 392-8.

14. Feher J, Deak G, Muzes G, Lang I., Niederland V, Nekam K et al. Liver protective action of silymarin therapy in chronic alcoholic liver diseases. Orv Hetil 1989; 130: 2723-7.

15. Pár A, Roth E, Rumi G, Kovács Z, Nemes J, Mózsik G. Oxidative stress and antioxidant defense in alcoholic liver disease and chronic hepatitis C. Orv Hetil 2000; 141: 1655-9.

16. Lucena MI, Andrade RJ, De la Cruz JP, Rodriguez Mendizabal M, Blanco E, Sanchez de la Cuesta F. Effects of silymarin MZ-80 on oxidative stress in patients with alcoholic cirrhosis. Results of a randomized, double-blind, placebo-controlled clinical study. Int J Clin Pharmacol Ther 2002; 40: 2-8.

17. Mayer KÉ, Myers RP, Lee SS. Silymarin treatment of viral hepatitis: a systematic review. J Viral Hepatitis 2005; 12: 559-67.

18. Tusenius KJ, Spoek JM, Kramers CW. Iscador Qu for chronic hepatitis C: an exploratory study. Complement Ther Med 2001; 9: 12-6.

19. Grossarth-Maticek R, Kiene H, Baumgartner SM, Ziegler R. Use of Iscador, an extract of European mistletoe (*Viscum album*), in cancer treatment: prospective nonrandomized and randomized matched-pair studies nested within a cohort study. Altern Ther Health Med 2001; 7: 57-66, 68-72.

20. Mansky PJ, Grem J, Wallerstedt DB, Monahan BP, Blackman MR. Mistletoe and gemcitabine in patients with advanced cancer: a model for the phase I study of botanicals and botanical-drug interactions in cancer therapy. Integr Cancer Ther 2003; 2: 345-52.

21. Happle R. Alternative medicine: really an alternative to academic medicine? Hautarzt 2000; 51: 439-43.

22. Paget GE, Barnes IM. Evaluation of drug activities. In: Laurence DR, Bacharach AL, editors. Toxicity tests. Vol. 1. London & New York: Academic Press; 1964. p.1-135.

23. Crowley LV. The Reitman-Frankel colorimetric transaminase procedure in suspected myocardial infarction. Clin Chem 1967; 13: 482-7.

24. Belfield A, Goldberg DM. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzyme 1971; 12: 561-73.

25. Tusenius KJ, Spoek AM, van Hattum J. Exploratory study on the effects of treatment with two mistletoe preparations on chronic hepatitis C. Arzneimittelforschung 2005; 55: 749-53.

26. Hajtó T, Hostanska K, Berki T, Pálinkás L, Boldizsár F, Németh P. Oncopharmacological perspectives of a plant lectin (Viscum album agglutinin-I): overview of recent results from in vitro experiments and in vivo animal models, and their possible relevance for clinical applications. Evid Based Complement Alternat Med 2005; 2: 59-67.

27. Van Huyen JP, Delignat S, Bayry J, Kazatchkine MD, Bruneval P, Nicoletti A et al. Interleukin-12 is associated with the in vivo anti-tumor effect of mistletoe extracts in B16 mouse melanoma. Cancer Lett 2006; 243: 32-7.

28. Bussing A, Bischof M, Hatzmann W, Bartsch F, Soto-Vera D, Fronk EM et al. Prevention of surgery-induced suppression of granulocyte function by intravenous application of a fermented extract from *Viscum album* L. in breast cancer patients. Anticancer Res 2005; 25: 4753-7.

29. Gray AM, Flatt PR. Insulin-secreting activity of the traditional antidiabetic plant *Viscum album* (mistletoe). J Endocrinol 1999; 160: 409-14.

30. Orhan DD, Aslan M, Sendogdu N, Ergun F, Yesilada E. Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album* subspecies (European mistletoe) in streptozotocin-diabetic rats. J Ethnopharmacol 2005; 98: 95-102.

31. Radenkovic M, Ivetic V, Popovic M, Mimica-Dukic N, Veljkovic S. Neurophysiological effects of mistletoe (*Viscum album* L.) on isolated rat intestines. Phytother Res 2006; 20: 374-7.

32. Pratt DS, Kaplan MM. Laboratory tests. In: Schiff ER, Sorrell MF, Maddrey WC, editors. Schiff's diseases of the liver. 8th ed. Vol. 1. Philadelphia: Lippincott-Raven; 1999. p.205-244.

33. Farghali H, Kamenikova L, Hynie S, Kmonickova E. Silymarin effects on intracellular calcium and cytotoxicity: a study in perfused rat hepatocytes after oxidative stress injury. Pharmacol Res 2000; 41: 231-7.

34. De La Puerta R, Martinez E, Bravo L, Ahumada MC. Effect of silymarin on different acute inflammation models and on leukocyte migration. J Pharm Pharmacol 1996; 48: 968-70.