International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 8 (3): 204-208, 2012
ISSN 1811-7775 / DOI: 10.3923/ijp.2012.204.208

Effects of the Mixture of *Melissa officinalis* L., *Cinnamomum zeylanicum* and *Urtica dioica* on Hepatic Enzymes Activity in Patients with Nonalcoholic Fatty Liver Disease

[1,5]Ali Akbar Malekirad, [2]Mohadeseh Mojtabaee, [3]Mahya Faghih,
[4]Gholamhassan Vaezi and [5]Mohammad Abdollahi
[1]Department of Biology, Payame Noor University, 19395-4697, Tehran, Iran
[2]Faculty of Medicine, Arak University of Medical Science, Arak, Iran
[3]Faculty of Medicine, Esfahan University of Medical Science, Esfahan, Iran
[4]Islamic Azad University (IAU), Semnan Branch, Iran
[5]Faculty of Pharmacy and Pharmaceutical Sciences Research Center,
Tehran University of Medical Science, Tehran, Iran

Abstract: Non-alcoholic fatty liver disease (NAFLD) is becoming one of the most common causes of chronic liver disease worldwide that does not have obvious effective drug. The aim of this study was to determine the capability of *Melissa officinalis* L. (Lemon balm), *Cinnamomum zeylanicum* and *Urtica dioica* mixture infusion (1.5, 0.5 and 0.25 g/100 mL) on patients with NAFLD. The study was designed as a before-after clinical trial and performed on 35 patients with NAFLD. They were asked to drink the mixture infusion which were prepared in special bags twice a day for 30 days. Liver markers of NAFLD such as alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphates (ALKp) in plasma were measured before and after using the infusion. The use of the infusion in NAFLD resulted in a significant decrease in ALT. Also activity of the AST and ALP were decreased after administration of the infusion but these decreases were not significant. Also a significant decrease in grade of sonographic examination was found. A significant linear correlation was also found between age and AST and ALT. However, no linear correlation was found between gender and weight and liver enzymes. Taken altogether, it is concluded that consumption of the present mixture has some benefits in NAFLD.

Key words: Non-alcoholic fatty liver disease, lemon balm, cinnamon, *Urtica dioica*, liver enzymes

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), first described in 1980, is presently recognized as one of the most common causes of elevated liver enzymes and chronic liver disease in Western countries (Stein *et al*., 2009). NAFLD happens among all age groups and societies and is recognized to occur in 14-30% of the general population (Adams and Angulo, 2006). NAFLD covers a wide spectrum of liver pathology from steatosis alone, through the necroinflammatory disorder of non-alcoholic steatohepatitis (NASH) to cirrhosis and liver cancer. NASH may be found in about one-third of such cases while 20-25% of NASH cases could end in cirrhosis (Raszeja-Wyszomirska *et al*., 2008). The pathogenesis of NASH is multifactorial, with insulin resistance and increased fatty acid possibly being important factors in the accumulation of hepatocellular fat, oxidative stress and mitochondrial dysfunction. Many pilot trials for antioxidants and lipid-lowering and hepatic protective agents have yielded promising initial results in improving liver enzymes or features of liver histology (Park, 2008). Both NAFLD and NASH are often discovered in overweight and obese patients with asymptomatic elevation of serum aminotransferase levels (Grattagliano *et al*., 2007). Antioxidants such as vitamin E, N-acetylcysteine, betaine and others may be useful in the treatment of NASH (Mehta *et al*., 2002; Rahimi *et al*., 2012). However, to keep the level of Reactive Oxygen Species (ROS) under control, living organisms have developed antioxidant systems that consisted of non-enzymatic ones such as glutathione, ascorbic acid, tocopherol, carotene, uric acid, bilirubin as well as enzymatic scavengers like superoxide dismutase (SOD), Glutathione Peroxidase (GPx) and catalase (CAT). Antioxidants can inhibit lipid peroxidation (LPO) by decreasing localized oxygen density, scavenging free radicals, stopping initiating radical generation,

Corresponding Author: Mohammad Abdollahi, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Science, Tehran 1417614411, Iran

decomposing peroxides and chain breaking to prevent continued hydrogen abstraction by active radicals. Studies in the recent years have proved applicability of natural and synthetic antioxidants in the management of many ailments including osteoporosis (Sharif *et al*., 2010), diabetes and islet transplantation (Hasani-Ranjbar *et al*., 2010a, b; Mohseni-Salehi-Monfared *et al*., 2009a; Momtaz and Abdollahi, 2010; Rahimi *et al*., 2005), inflammatory bowel diseases (Rezaie *et al*., 2007), preeclampsia (Rahimi *et al*., 2009) and pancreatitis (Mohseni-Salehi-Monfared *et al*., 2009b) and even in environmental toxicology (Fani *et al*., 2008; Abdollahi *et al*., 2004). In our previous studies, the antioxidant potential of Mellisa (Zeraatpishe *et al*., 2011) and Cinnamon (Fani *et al*., 2008) were reported. Also, *Urtica dioica* is known for its strong antioxidant properties in diabetes (Mehri *et al*., 2011). The antioxidant capacity of these herbal medicines were individually proved but not as a mixture. On the other hand, there is no proven specific therapy for NAFLD, hence the aim in the present research, was to explore beneficial effects of the mixture of Lemon balm, *Cinnamomum zeylanicum* and *Urtica dioica* on the patients with NAFLD.

MATERIALS AND METHODS

Plant material: The aerial parts of *Melissa officinalis* L. and *Urtica dioica* were collected in August 2011 from Botanical Garden of Arak University and identified as *Melissa officinalis* L. and *Urtica dioica* by Dr Salehi Arjmand from Department of Medicinal Plant, Faculty of Agriculture, Arak University. Cinnamon was supplied by Arak Medicinal Plants Company and identified as *Cinnamon Zeylanicum*. The leaves of *Melissa officinalis* L. and *Urtica dioica* were dried in shade at room temperature for 12 days.

Subjects: Thirty-five adults aged 26-71 years (15 men and 20 women) were randomly selected from patients who referred to the interdisciplinary ultrasound department of the Hospital of Shiraz University in Lar in the year 2011 for sonographic examination of the abdomen. After being recognized as belonging to the second stage of NAFLD, they were included in the study provided that they did not have a history of coronary artery disease, insulin-dependent diabetes, a bleeding diathesis, severe anemia, cancer within the past 5 years, any condition likely to lead to death within 5 years, use of anti-coagulants, cyclosporine and so none of the patients had suffered from viral hepatitis or autoimmune-related disorders or reported alcohol consumption. All participants were provided with specific written information about the aims of the study before written consents were obtained, in accordance with ethical rules of Pharmaceutical Sciences Research Center (PSRC) of Tehran University of Medical Science where the study protocol was approved. Prior to blood collection, each individual was extensively interviewed by a specialized physician who filled in a structured questionnaire about disease and habit diet. The included subjects were administered Lemon balm, Cinnamon and *Urtica dioica* mixture infusion (1.5, 0.5 and 0.25 g/100 mL) twice daily for 30 days at 7.5 am and 2 pm every day. Doses were obtained from our previous studies (Malekirad *et al*., 2011; Ranjbar *et al*., 2007; Mehri *et al*., 2011). A supervisor carefully checked to make sure that the volunteers were taking infusion properly. Blood samples were collected from all subjects before using mixture infusion and 12 h after the last dose of 30-day treatment with infusion.

Infusion preparation and protocol: Leaves of Lemon balm, *Urtica dioica* were dried and cleaned and then packed with Cinnamon in 1.5, 0.5 and 0.25 g bags. The subjects were instructed how to prepare the infusion by mixing a total of bags in 100 mL 98°C water for 30 min. A qualified expert supervised the whole procedure.

Biochemical analysis of serum parameters: All biochemical serum analyses were performed in the same laboratory including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Statistical analysis: Results are presented as Mean±SD. Statistical analyses were conducted using Stats Direct 2.7.8 software. The paired t-test and Wilcoxon Matched-Pairs Signed-Ranks Tests were applied. Relationships between parameters were determined by use of Pearson correlation analysis. The p-value of less than 0.05 was considered statistically significant.

RESULTS

The Mean±SD values for age and weight of subjects were 42.43±10.54 and 80.09±9.97, respectively. Of subjects, 15 (42.86%) were male and 20 (57.14%) were female.

After using the mixture infusion, ALT significantly ($p = 0.001$) reduced. The before and after Mean±SD were 133.49±76.65 and 114.08±66.98, respectively. A decrease in AST was observed after administration of infusion (119.43±154.18 before versus 87.8±68.46 after) but the decrease was not significant ($p = 0.17$). A decrease ($p = 0.12$) in ALP was observed by use of infusion. The

Table 1: The effect of infusion of Lemon balm, Cinnamon and *Urtica dioica* mixture on liver enzymes parameters

Enzyme activity	Before treatment	After treatment	p-value
ALT	133.49±76.65	114.08±66.98	0.001
AST	119.43±154.18	87.80±68.46	0.17
ALP	113.03±103.71	91.29±41.63	0.12

Table 2: Correlation between demographic factors and liver enzymes parameters

	AST		ALT		ALP	
Enzymes/demographic factor	r	p	r	p	r	p
Age	0.483	0.003	0.009	0.960	0.402	0.017
Weight	-0.43	0.160	0.311	0.084	0.068	0.713
Gender	-0.232	0.180	-0.147	0.399	-0.181	0.299

Mean±SD of before and after use of infusion were 113.03±103.71 and 91.29±41.63 (Table 1).

Analysis by Wilcoxon Matched-Pairs Signed-Ranks test showed a significant decrease in grade of sonographic examination ($p = 0.003$). A significant linear correlation was found between age and AST and ALT ($r = 0.48$, $p = 0.003$ and $r = 0.4$, $p = 0.017$, respectively) (Table 2). However, no linear correlation was found between gender and weight and liver enzymes.

DISCUSSION

In this study, the effect of Cinnamon, *Melissa officinalis* L. and *Urtica dioica* infusion on in NAFLD was tested and showed that the activity of ALT, AST and ALP decreased but only the decrease in ALT was statistically significant. Moreover, a significant linear correlation was found between age and AST and ALT. On the other hand, the ratio of AST/ALT after the treatment was decreased that is a good result.

Newly, the AST/ALT ratio greater than 1 is considered as a marker of disease severity (Clark, 2006). Moreover, NAFLD causes elevation of serum transaminase but ends up in fibrosis, cirrhosis and eventually hepatocellular carcinoma (Rodriguez *et al*., 2010). Of course, limited histological data support the association between improved aminotransferases and biopsy findings which require confirmation in a double-blind trial with appropriate statistical power based on liver histology (Bugianesi *et al*., 2005). The relationship between age and AST and ALT is reasonable since the rate of metabolic syndrome increases with age. Also, ALT is more specific to disorders of liver parenchyma cells.

Moreover, some studies reported that antioxidant therapy has beneficial effect on NAFLD or NASH. For example, one study indicated that Insulin sensitizing agents such as pioglitazone and anti-oxidant agents like vitamin E or IMOD® help improving liver histology in patients with NASH (Smith and Adams, 2011; Rahimi *et al*., 2012). Other antioxidants such as N-acetylcysteine, betaine, etc. were found beneficial in the treatment of NASH (Mehta *et al*., 2002). Although, many pilot trials for antioxidants and lipid-lowering and hepatoprotective agents have yielded promising initial results in improving liver enzymes or features of liver histology (Park, 2008) but some studies do not confirm benefit of antioxidant on reduction of ALT in patients with pediatric NAFLD (Lavine *et al*., 2011).

However, the role of antioxidants in protection of liver seems important. Many experimental studies show that *Urtica dioica* has a protective effect on the liver but human studies are rare (Mehri *et al*., 2011). Kandis *et al*. (2010) reported that *Urtica dioica* has a protective effect on the liver in hepatic ischemia-reperfusion-injured rats and histopathological examination showed that liver tissue damage was significantly decreased in *Urtica dioica* group in comparison to control group (Kandis *et al*., 2010). Moreover, other results indicated that ethanolic and water extracts have more potent hepatoprotective action (Moselhy and Ali, 2009). Other study showed that administration of *Urtica dioica* extracts can cause a little modulating in the main morphometric indices of liver such as area of hepatocytes, nuclei and nucleolus in periportal and perivenous zones (Golalipour *et al*., 2009). Ozkol *et al*. (2011) reported that almost all doses of *Urtica dioica* prevents toxicity of cisplatin by decreasing AST, ALT, as well as increasing the reduced glutathione content, SOD, CAT, glutathione S-transferase and GPx.

On the other hand, it was shown that Cinnamon act as a hepatoprotective and reduces serum ALT, AST in rats (Amin and Abd El-Twab, 2009). Cinnamon extract was found as a potent hepatoprotective that elevates serum AST and ALT in rat CCl_4-induced liver damage model (Moselhy and Junbi, 2010).

The effects of *Melissa officinalis* L. extract on hyperlipidemic rats has been also studied. The *Melissa officinalis* L. extract reduced serum total cholesterol, total lipid, ALT, AST and ALP levels and RES in liver tissue. Moreover, it increased glutathione levels in the tissue (Bolkent *et al*., 2005).

Taken together, it seems that the present mixture is full of phenolic compounds with a lot of scavenging properties that result in improvement of NAFLD.

CONCLUSION

The consumption of the present mixture is beneficial for NAFLD patients. Conducting multi-center clinical trials with higher sample size is recommended.

ACKNOWLEDGMENT

The authors would like to thank all subjects and authorities of the Grash Hospital in Lar for their kind cooperation.

REFERENCES

Abdollahi, M., A. Ranjbar, S. Shadnia, S. Nikfar and A. Rezaie, 2004. Pesticides and oxidative stress: A review. Med. Sci. Monit., 10: RA141-RA147.

Adams, L.A. and P. Angulo, 2006. Treatment of non-alcoholic fatty liver disease. Postgraduate Med. J., 82: 315-322.

Amin, K.A. and T.M. Abd El-Twab, 2009. Oxidative markers, nitric oxide and homocysteine alteration in hypercholesterolimic rats: Role of atorvastatine and cinnamon. Int. J. Clin. Exp. Med., 2: 254-265.

Bolkent, S., R. Yanardag, O. Karabulut-Bulan and B. Yesilyaprak, 2005. Protective role of *Melissa officinalis* L. extract on liver of hyperlipidemic rats: A morphological and biochemical study. J. Ethnopharmacol., 99: 391-398.

Bugianesi, E., E. Gentilcore, R. Manini, S. Natale and E. Vanni *et al*., 2005. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in non-alcoholic fatty liver disease. Am. J. Gastroenterol., 100: 1082-1090.

Clark, J.M., 2006. The epidemiology of nonalcoholic fatty liver disease in adults. J. Clin. Gastroenterol., 40: S5-S10.

Fani, A., A. Malekirad, I. Fani, H. Allahnazem, K. Rahzani, A. Ranjbar and S. Vosough-Ghanbari 2008. On the benefit of Cinnamonum Zeylanicum for radiology umit staff. J. Med. Sci., 8: 384-389.

Golalipour, M.J., S. Ghafari and M.H. Farsi, 2009. Effect of *Urtica dioica* L. extract on quantitative morphometric alterations of liver parenchymal cells in STZ diabetic rats. Int. J. Morphol., 27: 1339-1344.

Grattagliano, I., P. Portincasa, V.O. Palmieri and G. Palasciano, 2007. Managing nonalcoholic fatty liver disease: Recommendations for family physicians. Can. Family Physician, 53: 857-863.

Hasani-Ranjbar, S., H. Vahidi, S. Taslimi, N. Karimi, B. Larijani and M. Abdollahi, 2010a. A systematic review on the efficacy of herbal medicines in the management of human drug-induced hyperprolactinemia; potential sources for the development of novel drugs. Int. J. Pharmacol., 6: 691-695.

Hasani-Ranjbar, S., N. Nayebi, B. Larijani and M. Abdollahi, 2010b. A systematic review of the efficacy and safety of *Teucrium* species; from anti-oxidant to anti-diabetic effects. Int. J. Pharmacol., 6: 315-325.

Kandis, H., S. Karapolat, U. Yildirim, A. Saritas, S. Gezer and R. Memisogullari, 2010. Effects of *Urtica dioica* on hepatic ischemia-reperfusion injury in rats. Clinics, 65: 1357-1361.

Lavine, J.E., J.B. Schwimmer, M.L. Van Natta, J.P. Molleston and K.F. Murray *et al*., 2011. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: The TONIC randomized controlled trial. J. Am. Med. Assoc., 305: 1659-1668.

Malekirad, A.A., N. Hosseini, M. Bayrami, T. Hashemi, K. Rahzani and M. Abdollahi, 2011. Benefit of lemon verbena in healthy subjects, targeting diseases associated with oxidative stress. Asian J. Anim. Vet. Adv., 6: 953-957.

Mehri, A., S. Hasani-Ranjbar, B. Larijani and M. Abdollahi, 2011. A systematic review of efficacy and safety of *Urtica dioica* in the treatment of diabetes. Int. J. Pharm., 7: 161-170.

Mehta, K., D.H. Van Thiel, N. Shah and S. Mobarhan, 2002. Nonalcoholic fatty liver disease: Pathogenesis and the role of antioxidants. Nutr. Rev., 60: 289-293.

Mohseni-Salehi-Monfared, S.S., B. Larijani and M. Abdollahi, 2009a. Islet transplantation and antioxidant management: A comprehensive review. World J. Gastroenterol., 15: 1153-1161.

Mohseni-Salehi-Monfared, S.S., H. Vahidi, A.H. Abdolghaffari, S. Nikfar and M. Abdollahi, 2009b. Antioxidant therapy in the management of acute, chronic and post-ERCP pancreatitis: A systematic review. World J. Gastroenterol., 15: 4481-4490.

Momtaz, S. and M. Abdollahi, 2010. An update on pharmacology of *Satureja* species; from antioxidant, antimicrobial, antidiabetes and anti-hyperlipidemic to reproductive stimulation. Int. J. Pharmacol., 6: 454-461.

Moselhy, S.S. and H.H. Junbi, 2010. Antioxidant properties of ethanolic and aqueous Cinnamon extracts against liver injury in rats. Int. J. Adv. Pharm. Sci., 1: 151-155.

Moselhy, S.S. and H.K. Ali, 2009. Hepatoprotective effect of Cinnamon extracts against carbon tetrachloride induced oxidative stress and liver injury in rats. Biol. Res., 42: 93-98.

Ozkol, H., D. Musa, Y. Tuluce and I. Koyuncu, 2011. Ameliorative influence of *Urtica dioica* L against cisplatin-induced toxicity in mice bearing Ehrlich ascites carcinoma. Drug Chem. Toxicol., (In Press).

Park, S.H., 2008. Nonalcoholic steatohepatitis: Pathogenesis and treatment. Korean J. Hepatol., 14: 12-27.

Rahimi, H.R., M. Gholami, H.R. Khorram-Khorshid, F. Gharibdoost and M. Abdollahi, 2012. On the protective effects of IMOD and silymarin combination in a rat model of acute hepatic failure through anti oxidative stress mechanisms. Asian. J. Anim. Vet. Adv., 7: 38-45.

Rahimi, R., S. Nikfar, A. Rezaie and M. Abdollahi, 2009. A meta analysis on the efficacy and safety of combined vitamin C and E supplementation in preeclamptic women. Hypertens. Pregnancy, 28: 417-434.

Rahimi, R., S. Nikfar, B. Larijani and M. Abdollahi, 2005. A review on the role of antioxidants in the management of diabetes and its complications. Biomed. Pharmacother., 59: 365-373.

Ranjbar, A., S. Ghaseminejhad, H. Takalu, A. Baiaty, F. Rahimi and M. Abdollahi, 2007. Anti oxidative stress potential of cinnamon (*Cinnamomum zeylanicum*) in operating room personnel, a before/after cross sectional clinical trial. Int. J. Pharmacol., 3: 482-486.

Raszeja-Wyszomirska, J., M. Lawniczak, W. Marlicz, J. Miezynska-Kurtycz and P. Milkiewicz, 2008. Non-alcoholic fatty liver disease-new view. Pol. Merkur. Lekarski., 24: 568-571.

Rezaie, A., R.D. Parker and M. Abdollahi, 2007. Oxidative stress and pathogenesis of inflammatory bowel disease: An epiphenomenon or the cause. Dig. Dis. Sci., 52: 2015-2021.

Rodriguez, G., S. Gallego, C. Breidenassel, L.A. Moreno and F. Gottrand, 2010. Is liver transaminases assessment an appropriate tool for the screening of non-alcoholic fatty liver disease in at risk obese children and adolescents? Nutr. Hosp., 25: 712-717.

Sharif, P.S, M. Asalforoush, F. Ameri, B. Larijani and M. Abdollahi, 2010. The effect of n-3 fatty acids on bone biomarkers in Iranian postmenopausal osteoporotic women: A randomized clinical trial. AGE, 32: 179-186.

Smith, B.W. and L.A. Adams, 2011. Non-alcoholic fatty liver disease. Crit. Rev. Clin. Lab. Sci., 48: 97-113.

Stein, L.L., M.H. Dong and R. Loomba, 2009. Insulin sensitizers in nonalcoholic fatty liver disease and steatohepatitis: Current status. Adv. Therapy, 26: 893-907.

Zeraatpishe, A., S. Oryan, M.H. Bagheri, A.A. Pilevarian, A.A. Malekirad, M. Baeeri and M. Abdollahi, 2011. Effects of *Melissa officinalis* L. on oxidative status and DNA damage in subjects exposed to long-term low-dose ionizing radiation. Toxicol. Ind. Health, 27: 205-212.