Vol.57, n.3: pp. 340-348, May-June 2014
http://dx.doi.org/10.1590/S1516-89132014005000012
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Hepatoprotective Effects of Iranian *Hypericum scabrum* Essential Oils Against Oxidative Stress Induced by Acetaminophen in Rats

Abolfazl Dadkhah[1*], Faezeh Fatemi[2], Mohsen Eslami Farsani[3], Kambiz Roshanaei[4], Mahdi Alipour [4] and Hadi Aligolzadeh[5]

[1]Department of Medicine; Faculty of Medicine; Qom Branch; Islamic Azad University; Qom - I.R. - Iran. [2]Nuclear Fuel Cycle Research School; Nuclear Science and Technology Research Institute; Tehran - I.R. - Iran. [3]Department of Anatomy Science and Molecular Biology; School of Medicine; Isfahan University of Medical Sciences; Isfahan - Iran. [4]Department of Physiology; Faculty of Science; Qom Branch; Islamic Azad University; Qom - I.R. - Iran. [5]Azizi Hospital-Juybar; Mazandaran University of Medical Sciences; Sari - I.R - Iran

ABSTRACT

This studied examined the protective role of Hypericum scabrum *oils (100 and 200 mg/kg b.w, i.p) on acetaminophen-induced liver damages in the rat. The hepatic oxidative/antioxidant parameters such as lipid peroxidation (LP), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and ferric reducing ability of plasma (FRAP) were measured 2, 4, 8, 16 and 24h after the treatments confirmed by histopathological consideration. The results indicated that increased levels of hepatic LP and FRAP and SOD activity were reversed in the rats treated with oils. In addition, the depleted GSH were compensated with the oil treatments. The protective effect of the oils was further confirmed by the histophatological examination carried out on liver biopsies. The data pointed out that* H. scabrum *oil could modulate the hepatic toxicity induced by the APAP through adjusting the oxidative stress/antioxidant parameters and could be of potential candidate for the treatment of acetaminophen induced oxidative stress liver damages.*

Key words: *Hypericum scabrum*, essential oils, Acetaminophen, Rat, Oxidative stress, Liver

INTRODUCTION

Paracetamol or acetaminophen (APAP - acetyl-para-aminophenol) is a widely used over-the-counter pain reliever and fever reducer and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients (Boyer and Rouff 1971; Vermeulen et al. 1992). Paracetamol toxicity commonly occurred with acute overdoses is the foremost cause of liver damage (Larson et al. 2005; Khashab et al. 2007; Hawkins et al. 2007). There has been much interest in the possible contribution of oxidative stress to the initiation and/or progression of APAP-induced liver injury (Laskin et al. 1995; Michael et al. 1999; Sener et al. 2003; Dadkhah et al. 2006, 2007). Accordingly, as a vast clinical consumption of this drug, finding a way to reduce the side effects can have a great application potential.

Hypericum is a large genus of herbs or shrubs, which has more than 400 species used as traditional medicinal plants in various parts of the

[*]Author for correspondence: Dadkhah_bio@yahoo.com

world (Robson 1990; Yazaki and Okada 1994). In Iran, 19 species of five sections exist (Azadi 1999), which are generally known locally with the names "Hofarighon, Alafe chai and Gole raei" (Zargari 1985). Studies have indicated various therapeutic effects of this plant such as antidepressant like effect, antibacterial activity, management of cardiovascular disease, wound healing, antispasmodic and bronchodilator (Do Rego et al. 2007; Hakimoğlun et al. 2007; Süntar et al. 2010; Khan et al. 2011). Some studies have also indicated the insecticidal, larvicidal and antibacterial activities of *H. scabrum* extracts (Tozlua et al. 2011; Cetin et al. 2011; Akhbari et al. 2012). One study showed the protective ability of ethanol extracts of *H. scabrum L.* and *H. retusum Aucher* against the protein oxidation and DNA damage (Kızıl et al. 2011). *H. scabrum* aqueous extract showed remarkable antihypoxic and antidepressant effects (Eslami et al. 2011).

So, after considering the composition and antioxidant properties of *H. scabrum* essential oils *in vitro* system (Dadkhah et al. 2012), in this study, for the first time**,** the application of *H scabrum* essential oils in modulating the oxidative stress/antioxidant balance of APAP usage leading to liver injury was evaluated. The study also focused on hepatoprotection, as there has been an alarming increase in the incidence of APAP-related liver damage.

MATERIALS AND METHODS

Plant preparation

Fresh Iranian *H. scabrum* was collected in spring from the Alamut mountain in Qazvin province, Iran. The plant material was authenticated by Dr. Mozaffarian V. Oil extraction was carried out using a Clevenger-type apparatus and was analyzed by GC/MS analysis. Then, the radical scavenging and antioxidant activities of the oils were measured through DPPH and ß-Carotene-linoleic acid assays (Dadkhah et al. 2012).

Animal treatments

Male *Wistar* rats were used throughout this study. The animals were obtained from the Pasteur Institute of Iran and maintained in the animal house facilities. Adult animals were 3–4 months of age, weighing 180±20 g. They were maintained on a commercial pellet food and tap water *ad libitum*. The animals were divided into 16 groups (n=5). In negative control group (NC), the APAP vehicle, i.e., 400 µL DMSO was only injected. In control group (C), the acetaminophen (500 mg/kg b.w) dissolved in 400 µL DMSO was i.p injected. In the treatment groups, the essential oils prepared from the plants at two different doses, i.e., 100 and 200 mg/kg b.w were diluted in 400 µL DMSO and injected i.p immediately after acetaminophen administration. In positive control group, the BHT (10 mg/kg b.w) dissolved in 400 µL DMSO was injected i.p immediately after acetaminophen administration.

Preparation of tissue homogenate and plasma

The heparinated blood samples were collected at different time intervals (2, 4, 8, 16 and 24 h after APAP administration) by heart puncture from all the animals and centrifuged at 3000g for 10 min to obtain the plasma. Liver samples were immediately transferred to ice-cold containers and homogenized (20%, w/v) in the appropriate buffer using a homogenizer (E.L.M 2500). The homogenate was used to measure the biochemical parameters.

Biochemical assays

Lipid peroxidation

A weighed portion of the liver was homogenized in phosphate buffer (100 mM, pH 7.0) and used to measure the concentration of thiobarbituric acid reacting substances (TBARS) as an indicator of lipid peroxidation. The concentration of TBARS was measured spectrophotometrically according to the instruction of the kit purchased from Enzo Life Sciences, Inc., UK.

GSH estimation

GSH was estimated in liver homogenate based on the protocol of the purchased kit from BioVision, Inc., USA.

Determination of SOD and CAT enzyme activities

The activities of SOD and CAT were estimated in liver homogenate using the commercial kits (BioVision, Inc., USA), following the instructions given by the company.

Ferric reducing ability of plasma (FRAP) assay

This assay was performed using TPTZ reagent as described by Benzie and Strain (1996). FRAP level was calculated by plotting a standard curve of absorbance against µmol/L concentration of Fe (II) standard solution.

Histophatological studies

The histological changes were quantitatively analyzed by a veterinary pathologist. All the animals (n=25) were sacrificed 24 h after acetaminophen administrations. Small portions of liver were excised from the central lobe and were fixed in 10% buffered formaldehyde solution, embedded in paraffin and sectioned at 6 micrometers. Then, the samples stained with hematoxylin and eosin (H&E) and studied with light microscope for histological analysis. The samples were obtained from each rat in triplicate (n=75). In each slide, four zones were considered randomly and the mean value was reported.

Statistical analysis

Data are presented as means ± Standard Error of Mean (SEM) of five samples obtained from five animals in each group. The results were subjected to one-way ANOVA followed by Tukey's HSD using SPSS (version 19.0) software. Significant levels were defined as $P<0.05$. (*) denote significantly different from the respective negative control group ($P < 0.05$). (**) denote significantly different from the respective control group ($P < 0.05$).

RESULTS

Effects of *H. scabrum* essential oil on the plasma and hepatic oxidative/antioxidant injury parameters in the rats treated by APAP

As shown in Figures 1-5, the oxidative/ antioxidant parameters, i.e., LP, GSH, SOD and CAT were analyzed in time dependent manner at 2, 4, 8, 16 and 24h after APAP administration. The data indicated that LP level was increased significantly only 4h after administration as compared to negative control group ($P<0.05$). The APAP administration caused the liver GSH depletion only at 4 and 8h after the treatment ($P<0.05$). The SOD activity was significantly increased at 8h after administration and remained in high level until 24h ($P<0.05$). The CAT activity was not significantly changed in all time intervals ($P>0.05$). As shown in Figure 5, the FRAP value was significantly higher in APAP-treated rats at 4h as compared to negative control group ($P<0.05$).

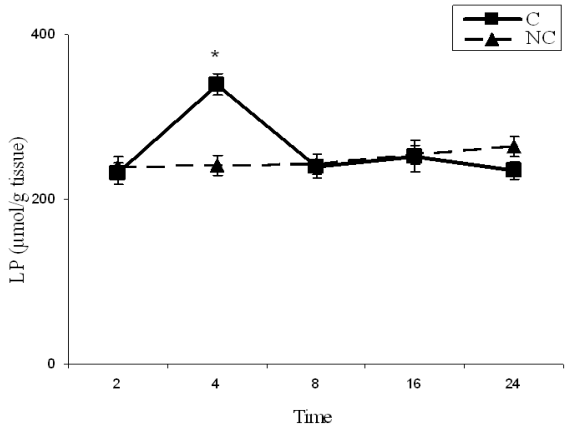

Figure 1 - Time-course changes in LP levels in rats treated with APAP in compare to negative controls. NC: Negative control group; C: Control group.

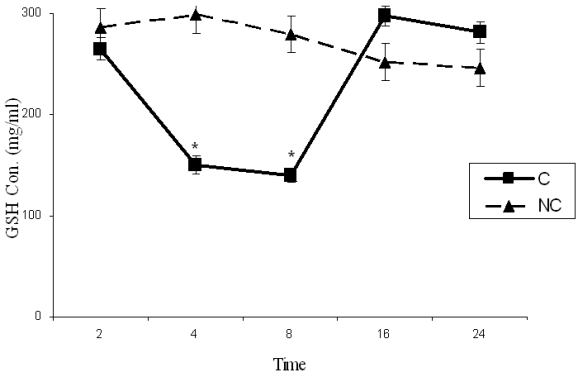

Figure 2 - Time-course changes in GSH levels in rats treated with APAP in compare to negative controls. NC: Negative control group; C: Control group.

From the data in Figures 1-5, it was deduced that APAP administration in time intervals (2, 4, 8, 16 and 24h) could only change the LP level at 4h, GSH level at 4 and 8h, SOD activity at 8-24h and FRAP level at 4h. Hence, considering the effects of i.p administration of *H. scabrum* oil on the levels of oxidative plasma and liver injury parameters in the experimental treated rats should be done only at 4h after acetaminophen administration as presented in Figures 6, 7, 10 and 11.

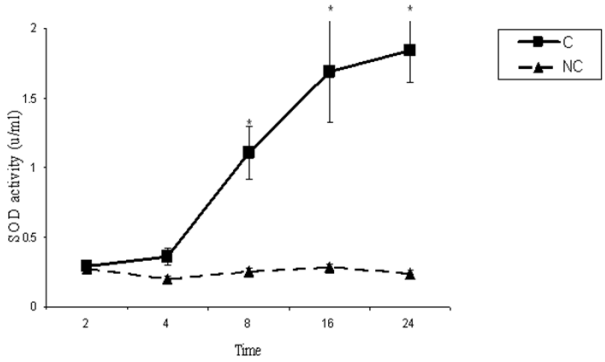

Figure 3 - Time-course changes in SOD activities in rats treated with APAP in compare to negative controls. NC: Negative control group; C: Control group.

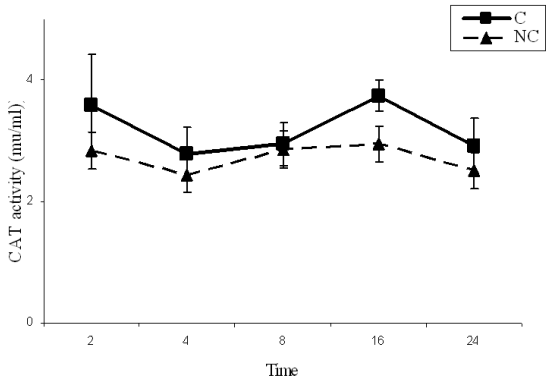

Figure 4 - Time-course changes in CAT activities in rats treated with APAP in compare to negative controls. NC: Negative control group; C: Control group.

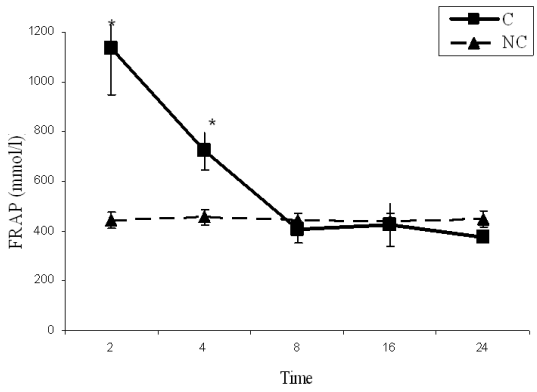

Figure 5 - Time-course changes in FRAP levels in rats treated with APAP in compare to negative controls. NC: Negative control group; C: Control group.

As the changes of parameters such as SOD activity and the GSH level were also shown in 8h after APAP administration, related treatment groups with essential oil were considered at 8h too (Figs. 8 and 9). In fact, the above parameters in all groups in the entire time intervals were investigated, but not shown here.

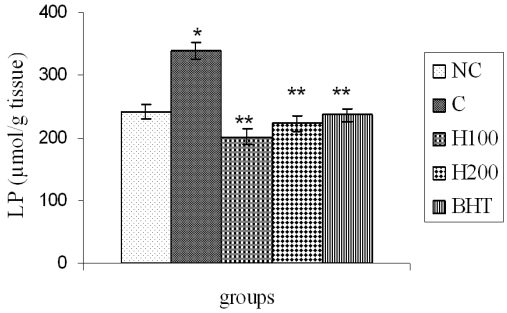

Figure 6 - Effect of *Hypericum scabrum* essential oil on LP levels 4h after APAP administration. NC: Negative control group; C: Control group; H100: *H. scabrum* essential oil (100mg/kg b.w); H200: *H. scabrum* essential oil essential oil (200mg/kg b.w); BHT: Butylated hydroxytoluene.

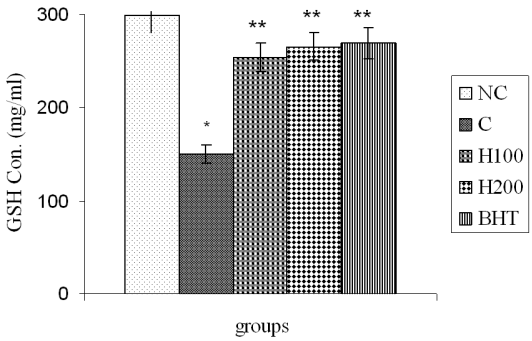

Figure 7 - Effect of *Hypericum scabrum* essential oil on GSH levels 4h after APAP administration. NC: Negative control group; C: Control group; H100: *H. scabrum* essential oil (100mg/kg b.w); H200: *H. scabrum* essential oil essential oil (200mg/kg b.w); BHT: Butylated hydroxytoluene.

The data indicated that as reference antioxidant (BHT), the plant essential oil at both doses of 100 and 200 mg/kg b.w. could decrease the LP to control level ($P<0.05$) (Fig. 6). The GSH level could successfully reach to normal level both at 4 and 8h after essential oil and BHT treatments as compared to the control groups ($P<0.05$) (Figs. 7 and 8).

The SOD activity also returned to normal level after administration of the plant essential oil at both doses as also seen in the positive control group at 8h after the treatment (P<0.05) (Fig. 9). However, no difference was noticed in the CAT activity in all the groups (P>0.05) (Fig. 10). Administration of the essential oil to APAP-treated rats had no effects on FRAP level at 4h (P>0.05) (Fig. 11).

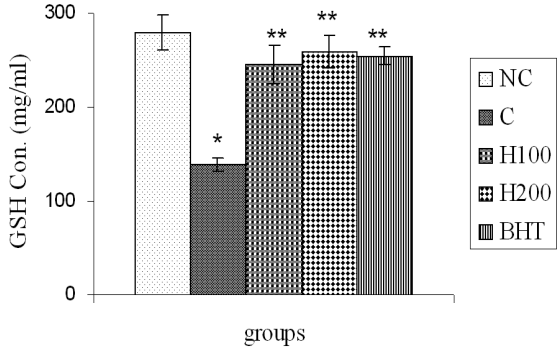

Figure 8- Effect of *H. scabrum* essential oil on GSH levels 8h after APAP administration. NC: negative control group; C: Control group; H100: *H. scabrum* essential oil (100 mg/kg b.w); H200: *H. scabrum* essential oil essential oil (200 mg/kg b.w); BHT: Butylated hydroxytoluene.

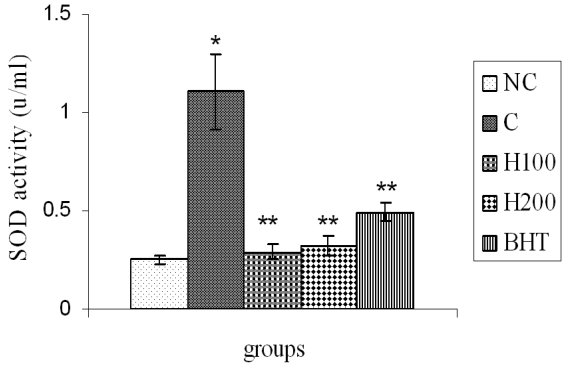

Figure 9 - Effect of *H. scabrum* essential oil on SOD activities 8h after APAP administration. NC: Negative control group; C: Control group; H100: *H. scabrum* essential oil (100 mg/kg b.w); H200: *H. scabrum* essential oil essential oil (200 mg/kg b.w); BHT: Butylated hydroxytoluene.

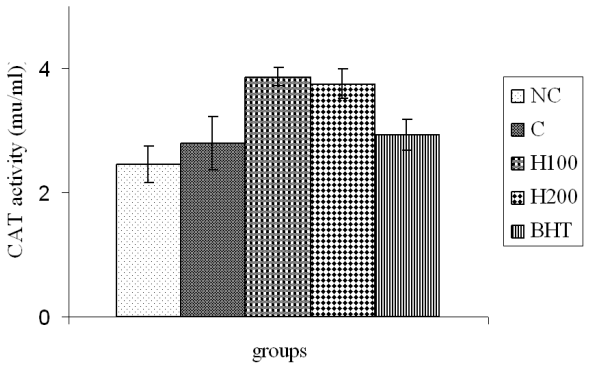

Figure 10 - Effect of *Hypericum scabrum* essential oil on CAT activities 4h after APAP administration. NC: Negative control group; C: Control group; H100: *H. scabrum* essential oil (100mg/kg b.w); H200: *H. scabrum* essential oil essential oil (200mg/kg b.w); BHT: Butylated hydroxytoluene.

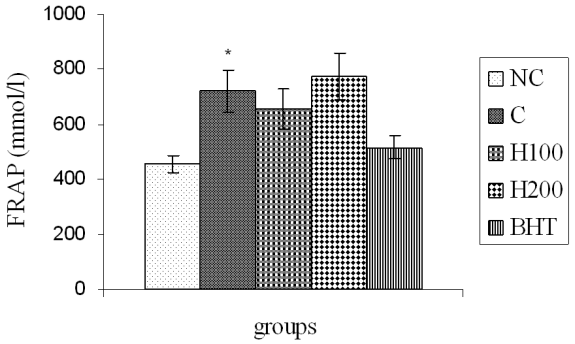

Figure 11 - Effect of *Hypericum scabrum* essential oil on FRAP levels 4h after APAP administration. NC: Negative control group; C: Control group; H100: *H. scabrum* essential oil (100mg/kg b.w); H200: *H. scabrum* essential oil essential oil (200mg/kg b.w); BHT: Butylated hydroxytoluene.

Effects of *Hypericum scabrum* essential oil on hepatic histophatological changes in rats treated by APAP

Histopathological studies performed on liver biopsies showed normal structure of liver tissue in negative control group (Fig. 12A). In liver tissue of the rats treated with acetaminophen, minimal 3-8 points of hyalinized cells were detected. In some of the cells, wholly white, hyalinized, karyokisis and chromatolysis cytoplasm was also observed. Occasionally, these hyalinized aggregations seemed in one parallel row near together affected zonation and distribution of the blood in the liver

(Fig. 12B). The necrotic aggregations detected in liver of rats treated with acetaminophen together with 100mg/kg b.w of *H. scabrum* essential oils were less than those in the control group. The histological structures of sinusoids, hepatocytes, veins, arteries and portal spaces were relatively normal (Fig. 12C). No necrotic aggregation was observed in the rats treated with acetaminophen plus 200 mg/kg b.w. of *H. scabrum* essential oil. The sinusoids were some more wide and hepatocytes seemed more basophilic. There was little histological distributions (Fig. 12D) but no effect of necrotic aggregation in the rats treated with acetaminophen plus BHT was observed. The histological structures of sinusoids, hepatocytes, veins, arteries and portal spaces were perfectly normal (Fig. 12E).

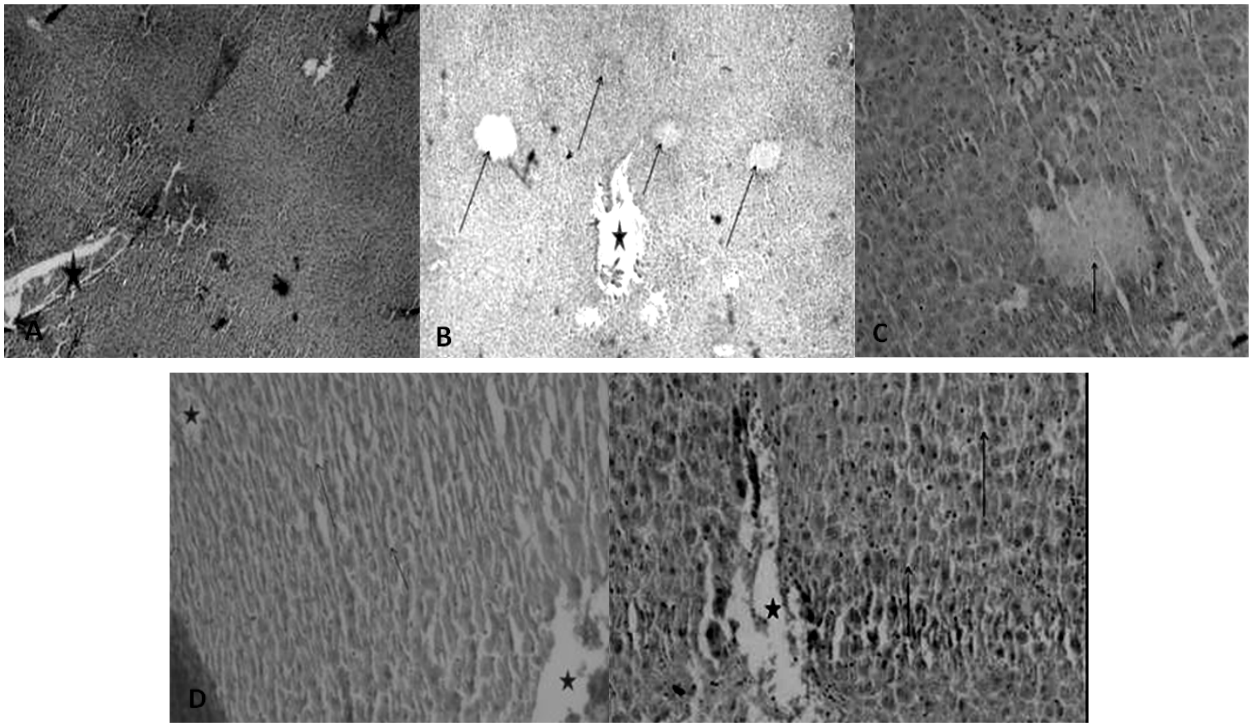

Figure 12 - Effect of *Hypericum scabrum* essential oil on histopathological changes 24h after APAP administration.

Light microscopy showed histological sections of the rat livers from different groups. A: Negative control group with normal structure of liver tissue and normal Central vein (stars) (original magnification×40). B: Control group with necrotic hyalinized aggregation (arrow) (original magnification×40). C: Treatment group (100 mg/kg b.w oils), cells of necrotic zone (arrow) are pale and more hyalinized in compare to aside hepatocyte cells (original magnification×100). D: Treatment group (200 mg/kg b.w oils), sinusoids are some more width and hepatocytes seem more basophilic without any necrotic aggregation. Some little histological distributions were observed (original magnification×100). E: Positive control group (10 mg/kg b.w BHT), sinusoids (arrows) and hepatocytes around of central vein are normal (star) without any necrotic zone (original magnification×100).

DISCUSSION

Recently, it has been shown that α-pinene (40.9%), spathulenol (7.9%), β-pinene (5.2%), α-cadinol (4.7%), limonene (4.3%) and *epi-α*-muurolol (3.2%), identified with GC/GC–MS analysis, constituted the major compounds of *H. scabrum* essential oils with potent *in vitro* antioxidant activities (Dadkhah et al. 2012). Continuing the *in vivo* effectiveness, this study showed that *H. scabrum* essential oil could modulate the

oxidative/antioxidant statues, leading to condensing the acetaminophen-induced hepatic toxicity. This was confirmed by the histopathological tests, which showed decrease of necrotic cell by the essential oil in acetaminophen treated rats. These data increase the understanding about the mechanisms by which the essential oil affected the APAP -induced liver toxicity.

The low level of hepatic antioxidant parameter (GSH) in APAP-treated rats with simultaneous development of hepatic injury (Figs. 2 and 12b) together with increased LP level (Fig. 1) indicated that the oxidant-antioxidant homeostasis was disturbed in APAP-treated rats due to acetaminophen metabolisms and also the liver was susceptible to oxidative damage during APAP metabolism (Dadkhah et al. 2006; Shanmugasundaram and Venkataraman 2006; Dadkhah et al. 2007; Kumari et al. 2012). Liver is the major site of detoxification and the primary target of drug exposure in the body. High levels of drugs cause various hepatic disorders by producing pro-oxidants/reactive oxygen species (ROS), which are able to induce cellular damage in a variety of ways by affecting the cellular biomolecules, such as lipids, DNA and proteins (Ziech et al. 2010). It has been further suggested that the generation of ROS during the metabolic activation of paracetamol in the CYP450 system (Dai and Cederbaum 1995) and from the mitochondria during paracetamol intoxication (Knight et al. 2001) appear as an early event, which precedes intracellular GSH depletion (Fig. 2) and cell damage in paracetamol hepatotoxicity (Manov et al. 2002). The activity of SOD is a sensitive index in hepatic damage as it scavenges the superoxide anion to form hydrogen peroxide, leading to diminish the toxic effects (Bhadauria and Nirala 2009). Changes in the activity of this antioxidant enzyme (Fig. 3) might occur as the result of imbalance between the prooxidant and antioxidant systems in the liver.

Increased TBARS level in the APAP-treated rats (Fig. 1) indicated the main role of LP in the initiation of oxidative stress. Excessive ROS generation triggers the process of lipid peroxidation in cell membranes and causes the destruction of cell components and cell death. Lipid peroxidation and protein carbonyl content are two important parameters to assess the oxidative damage to lipids and proteins, respectively. Earlier studies have shown that hepatic lipid peroxidation increases during the APAP toxicity (James et al. 2003; Dadkhah et al. 2006; Sharma et al. 2011). The induction of the FRAP in plasma and SOD activity in liver of the APAP treated rats (Figs. 3 and 5) could be due to the compensatory increased of enzymatic and non-enzymatic antioxidants, which led to increased resistance and/or decreased susceptibility of the liver to free radical attack (Manju and Nalini 2005; Dadkhah et al. 2006). These changes were accompanied by no change in the CAT activity (Fig. 4), implying the imbalance among the antioxidants, resulting to the accumulation of toxic-free radicals that caused cell damage (Sun 1990). Other studies also indicated the disturbed oxidative stress/antioxidant statues by APAP involved in hepatotoxicity (Shanmugasundaram and Venkataraman 2006; Kumari et al. 2012).

As shown in Fig. 6-11, *H. scabrum* essential oil (100 and 200 mg/kg b.w.) could significantly modulated the oxidative stress/antioxidant parameters, i.e., SOD, GSH, FRAP and LP. The essential oil significantly restored the hepatic GSH level, showing the oil could scavenge the reactive free radicals that eventually reduced the oxidative damage to the tissues and subsequently improved the level of this antioxidant (Bhadauria and Nirala 2009). In addition, the SOD activity and LP and FRAP levels, as important factors in the oxidative stress/antioxidant balancing (Dadkhah et al. 2006) was compensated by the essential oil. These cellular oxidative/antioxidant factors play an important role in maintaining the redox homeostasis under normal physiological conditions. APAP treatment depleted the glutathione level and caused oxidative stress and redox imbalance. The essential oil treatment significantly prevented the decline in the activities of the oxidative stress/antioxidant parameters altered due to APAP administration (Kumari et al. 2012).

One study also confirmed these results indicating Lupeol, a triterpene, when co-administered with APAP effectively reduced the oxidative stress and prevented the APAP-induced hepatotoxicity by inhibiting the critical control points of apoptosis (Kumari et al. 2012). Another study reported the protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March, trunk wood resin, against acetaminophen-induced liver injury in mice through modulating the oxidative stress/antioxidant parameters (Oliveira et al. 2005). The protective effects of quercetin and curcumin on paracetamol-induced

liver injury in the rat through modulating the oxidative stress/ antioxidant parameters has also been reported (Yousef et al. 2010). The protective effect of *Aquilegia vulgaris* (L.) on APAP-induced oxidative stress in rats was mediated by amelioration of microsomal lipid peroxidation and restoring the antioxidant enzymes activity (Jodynis-Liebert et al. 2005). These data were confirmed by histopathological examinations (Fig. 12), indicating that the administration of essential oil decreased the oxidative hepatic injuries such as necrotic aggregations in acetaminophen treated rats.

CONCLUSION

Results showed that *H. scabrum* essential oil could modulate the hepatic toxicity induced by the APAP through adjusting the oxidative stress/antioxidant parameters, i.e., SOD, GSH, FRAP and LP confirmed by the histopathological findings. This study supported the traditional use of *H. scabrum* as a medicinal agent and suggested the feasibility of developing herbal drugs for the treatment of liver disorders.

REFERENCES

Akhbari M, Batooli H, Mozdianfard M. Comparative study of composition and biological activities of SDE prepared essential oils from flowers and fruits of two Hypericum species from central Iran. *Nat Prod Res*. 2012; 26: 193-202.

Azadi R. Guttiferae. In: M. Assadi (ed), *Flora of Iran*, Research Institute of Forests and Rangelands, Tehran. 1999; Vol. 27: pp. 4, 7, 22-33, 44-47.

Bhadauria M, Nirala SK. Reversal of acetaminophen induced subchronic hepatorenal injury by propolis extract in rats. *Environ Toxicol Pharmacol.* 2009; 27: 17-25.

Boyer TD, Rouff LJ. Acetaminophen-induced hepatic necrosis and renal failure. *J Am Med Assoc.* 1971; 218: 440-441.

Cetin H, Yanikoglu A, Cilek JE. Larvicidal activity of selected plant hydrodistillate extracts against the house mosquito, Culex pipiens, a West Nile virus vector. *Parasitol Res.* 2011; 108: 943-8.

Dadkhah A, Allameh AA, Fatemi F, Rasmi Y, Ashrafihelan J. Considering the pathologic lesions of liver and changes of plasma alanine transaminase and aspartate transaminase in acetaminophen–induced toxicity in rat. *Pharm Sci*. 2007; 2: 47-54.

Dadkhah A, Fatemi F, Kazemnejad S, Rasmi Y, Ashrafi-Helan J, Allameh A. Differential effects of acetaminophen on enzymatic and non-enzymatic antioxidant factors and plasma total antioxidant capacity in developing and adult rats. *Mol Cell Biochem.* 2006; 281: 145-152.

Dadkhah A, Roshanaei K, Fatemi F, Kazemi M , Alipour M, Abdolmohammadi MH. Chemical composition and antioxidant properties of Iranian Hypericum Scabrum Extracts From Alamut Mountain. *JEOBP*. 2014. In Press ID: 764193 DOI:10.1080/0972060X.2013.764193

Dai Y, Cederbaum AI. Cytotoxicity of acetaminophen in human cytochrome P4502E1-transfected HepG2 cells. *J Pharmacol Exp Ther*. 1995; 273: 1497-1505.

Do Rego JC, Benkiki N, Chosson E, Kabouche Z, Seguin E, Costentin J. Antidepressant-like effect of hyperfoliatin, a polyisoprenylated phloroglucinol derivative from *Hypericum perfoliatum* (Clusiaceae) is associated with an inhibition of neuronal monoamines uptake. *Eur J Pharmacol*. 2007; 569: 197-203.

Eslami B, Nabavi SF, Nabav SM, Ebrahimzadeh MA, Mahmoudi M. Pharmacological activities of *Hypericum scabrum* L. *Eur Rev Med Pharmacol Sci.* 2011; 15: 532-537.

Hawkins LC, Edwards JN, Dargan PI. Impact of restricting paracetamol pack sizes on paracetamol poisoning in the United Kingdom: a review of the literature. *Drug Saf*. 2007; 30(6): 465-479.

Hakimoğlu F, Kızıl G, Kanay Z, Kızıl M, Hilmi I. The effect of ethanol extract of *Hypericum lysimachioides* on lipid profile in hypercholesterolemic rabbits and its *in vitro* antioxidant activity. *Atherosclerosis.* 2007; 192: 113-122.

James LP, Mayeux PR, Hinson JA. Acetaminophen induced hepatotoxicity. *Drug Metab Dispos.* 2003; 31:1499-1506.

Jodynis-Liebert J, Matławska I, Bylka W, Marek Murias M. Protective effect of *Aquilegia vulgaris* (L.) on APAP-induced oxidative stress in rats. *J Ethnopharmacol.* 2005; 97 (2): 351-358.

Khashab M, Tector AJ, Kwo PY. Epidemiology of acute liver failure. *Curr Gastroenterol Rep*. 2007; 9(1): 66-73.

Khan A, Gilani A.H, Rehman N. Pharmacological studies on *Hypericum perforatum* fractions and constituents. *Pharm Biol*. 2011; 49: 46-56

Kızıl Go, Kızıl M, Ceken B, Yavuz M, Demir H. Protective Ability of Ethanol Extracts of *Hypericum scabrum* L. and Hypericum Retusum Aucher Against the Protein Oxidation and DNA Damage. *Int J Food Prop.* 2011; 14: 926-940.

Knight TR, Kurtz A, Bajt ML, Hinson JA, Jaeschke H. Vascular and hepatocellular peroxynitrite formation during acetaminophen-induced liver. injury: Role of mitochondrial oxidant stress. *Toxicol Sci*. 2001; 62: 212-220.

Kumari A, Kakkar P. Lupeol prevents acetaminophen-induced in vivo hepatotoxicity by altering the Bax/Bcl-2 and oxidative stress-mediated mitochondrial signaling cascade. *Life Sci*. 2012; 90: 561-570.

Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO, Lee WM; Acute Liver Failure Study Group. Acute Liver Failure Study Group. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology*. 2005; 42:1364-1372.

Laskin DL, Gardner CR, Price VF, Jollow DJ. Modulation of macrophage functioning abrogates the acute hepatotoxicity of acetaminophen. *Hepatology*. 1995; 21: 1045-1050.

Manju V, Nalini N. Chemopreventive efficacy of ginger, a naturally occurring anticarcinogen during the initiation, post-initiation stages of 1,2 dimethylhydrazine-induced colon cancer. *Clin Chim Acta*. 2005; 358: 60-67.

Manov I, Hirsh M, Lanccu TC. Acetaminophen hepatoxicity and mechanisms of its protection by N-acetylcysteine: A study of Hep3B cells. *Exp Toxicol Pathol*. 2002; 53: 489-500.

Michael SL, Pumfod NR, Mayeux PR, Niesman MR, Hinson JA. Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. *Hepatology*. 1999; 30: 186-195.

Oliveira FA, Chaves MH, Almeida FR, Lima RC Jr, Silva RM, Maia JL, et al. Protective effect of α- and β-amyrin, a triterpene mixture from Protium heptaphyllum (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J Ethnopharmacol*. 2005; 98 (1-2): 103-108.

Robson NKB. Studies in the genus Hypericum L. (Guttiferae) 8. Section 29. Brathys (part 2) and 30. Trigynobrathys. Bulletin of the British Museum-Natural History: Botany. 1990; 20: 1-151.

Sener G, Ozer A, Sehirli, Dulger A. Protective effects of melatonin, vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: A comparative study. *J Pineal Res*. 2003; 35: 61-68.

Sharma S, Singh RL, Kakkar P. Modulation of Bax/Bcl-2 and caspases by probiotics during cetaminophen induced apoptosis in primary hepatocytes. *Food Chem Toxicol*. 2011; 49:770-779.

Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) Heine Acanthaceae root extract. *J Ethnopharmacol*. 2006; 104(1-2): 124-128.

Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radical Biol Med*. 1990; 8: 583-599.

Süntar IP, Akkol EK, Yilmazer D, Baykal T, Kirmizibekmez H, Alper M, Yeşilada E. Investigations on the *in vivo* wound healing potential of *Hypericum perforatum* L. *J Ethnopharmacol*. 2010; 127: 468-477.

Tozlua E, Cakirb A, Kordalic S, Tozluc G, Ozerd H, Akcine TA. Chemical compositions and insecticidal effects of essential oils isolated from *Achillea gypsicola, Satureja hortensis, Origanum acutidens* and *Hypericum scabrum* against broadbean weevil (*Bruchus dentipes*). *Sci Hortic*. 2011; 130: 9-17.

Vermeulen NPE, Bessems JGM, Van De Straat. Molecular aspect of paracetamol - induced hepatotoxicity and its mechanism based prevention. *Drug Metabol Rev*. 1992; 24: 367-407.

Yazaki K, Okada T. Medicinal and Aromatic Plants VI. In: Bajaj, Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry, vol. 26. Springer-Verlag Berlin, 1994; pp. 167-178.

Yousef MI, Omar SAM, El-Guendi MI. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food Chem Toxicol*. 2010; 48: 3246-3261.

Zargari A. Medicinal plants, Tehran University, Tehran, Iran. 1985; 1: 125-340.

Ziech D, Franco R, Georgakilas AG, Georgakila S, Malamou-Mitsi V, Schoneveld O, Pappa A, Panayiotidis MI. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. *Chem Biol Interact*. 2010; 188: 334-339.

Received: April 28, 2013;
Accepted: January 13, 2014.