in vivo *31*: 209-214 (2017)
doi:10.21873/invivo.11047

The Effect of Geraniol on Liver Regeneration After Hepatectomy in Rats

MEDIHA CANBEK[1], MUSTAFA UYANOGLU[1], SELCUK CANBEK[2], EMRE CEYHAN[1], AHMET OZEN[1], BASAK DURMUS[1] and OZGE TURGAK[1]

[1]*Department of Biology, Faculty of Science, and* [2]*Department of Computer Engineering, Faculty of Engineering and Architecture, Eskisehir Osmangazi University, Eskisehir, Turkey*

Abstract. *Geraniol is a monoterpenoid alcohol that has a hepatoprotective effect. We investigated the regenerative effects of geraniol in rats after a 70% partial hepatectomy (PH). Using Wistar albino rats, nine groups were created: Group I was the control group, while the remaining groups received a single intraperitoneal dose of saline, Silymarin, or geraniol after PH. A 70% PH was performed on all groups except for groups II and III. Blood serum samples were obtained for alanine amino transferase (ALT) analysis. Then liver tissues were harvested for histological and real-time polymerase chain reaction (PCR) analyses. Tumor necrosis factor-α (TNFα) and interleukin 6 (IL6) gene expression were examined 24 and 48 h after PH. ALT levels were found to be statistically significantly increased in all PH-treated groups. TNFα and IL6 gene expression levels were elevated in geraniol-treated groups. Histological evaluation revealed a hepatoprotective effect for geraniol-treated groups. Our results suggest that geraniol plays a significant role during liver regeneration, which involves the elevated expression of TNFα and IL6 48 h after PH.*

Liver damage or a partial hepatectomy (PH) of the liver increases mitotic activity in the liver. Such regeneration continues until the hepatic lobes reach mature dimensions. Studies of rat liver show that in approximately 70% of cases, mitosis begins 24-30 h after hepatectomy (1-4).

Hepatic regeneration occurs through enzymatic and humoral mechanisms that involve hormones, growth factors, cytokines, activators, and inhibitors (5, 6). In the first few hours following hepatectomy, many genes related to the cytokine network are induced, and mRNA levels increase for tumor necrosis factor-α (TNFα) and interleukin-6 (IL6) (7, 8).

Many factors influence hepatic regeneration, including age, portal circulation, vitamins and infection, among others. In addition, various studies have identified chemical substances (anti-oxidants, *etc.*) that have positive effects on hepatic regeneration (9, 10).

Geraniol, a monoterpenoid alcohol, is an oily, pale yellow liquid (11-13) found among the volatile oils of plants such as roses, geraniums, carnations, and lavender (12, 13). Geraniol is an effective anti-oxidant, and a study has demonstrated the protective effects of geraniol on liver (14). However, as far as we are aware there are no studies of geraniol and liver regeneration.

Here, we used a rat model for investigating the effects of geraniol on *TNFα* and *IL6* gene expression 24 and 48 h after PH.

Materials and Methods

The experimental protocols for this study were approved by the Institutional Ethical Committee for Animal Care and Use at Eskisehir Osmangazi University, Eskisehir, Turkey. Animals were obtained from the medical and surgical experimental research center at our institute, and all experiments were carried out at this same center (protocol number: 270/2012).

Animals. Male Wistar albino rats with a body weight of 200-250 g were obtained from the Ministry of Health, Refik Saydam National Public Health Agency, and Experimental Animals Production Laboratory, Turkey. The experiment was performed following a period of adaption to the laboratory. The animals were housed in polycarbonate cages in an air-conditioned room with a 12/12-h light/dark cycle, temperature of 22±2˚C, and 50±5% humidity. Throughout the experimental period, the animals were provided with pellet chow and water *ad libitum*.

Experimental design. Animals were divided into the following nine groups, each consisting of 10 animals: Group I: control group, sacrificed at 0 h; groups II and III: sham-operated control groups sacrificed at 24 and 48 h, respectively; groups IV and V: PH saline-

This article is freely accessible online.

Correspondence to: Mediha Canbek, Department of Biology, Faculty of Science, Eskisehir Osmangazi University, 26480 Eskisehir, Turkey. Tel: +90 2222393750 ext. 2849, Fax: +90 2222393578, e-mail: mcanbek@ogu.edu.tr

Key Words: ALT, IL6, monoterpenoid alcohol, RT-PCR, TNFα.

treated groups sacrificed at 24 and 48 h, respectively; groups VI and VII: PH Silymarin-treated groups, which were positive control group sacrificed at 24 and 48 h, respectively; groups VIII and IX: PH geraniol-treated groups sacrificed at 24 and 48 h, respectively.

Chemicals. We used 99% pure geraniol (Acros Organics Thermo Fisher Scientific Geel, Belgium). Silymarin (Sigma-Aldrich Chemie, Taufkirchen Germany), which has protective and reparative effects on liver, was used as a positive control for regeneration. Geraniol and Silymarin were dissolved in saline solution, and a single intraperitoneal 100 mg/kg dose was given to the experimental animals (16, 17).

Surgical procedure. All surgical procedures were performed under anesthesia with 10 mg/kg xylazine and 70 mg/kg ketamine, injected intramuscularly. PH was performed using the Higgins and Anderson technique (18). All rats were housed with a 12-h light/dark schedule and allowed access to food and water *ad libitum*. In the rat 70% PH model, the median and left lobes were resected from male rats. The remaining liver lobes were obtained from defined time points after PH for biochemical, histological and molecular analysis.

Real-time polymerase chain reaction (PCR) assay. RNA isolation from liver tissue was performed using PureLink RNA Mini Kit and Trizol (Applied Biosystems, Paisley, UK), according to the kit protocol. cDNA was reverse transcribed from RNA using the High-Capacity cDNA Kit (Applied Biosystems). For this experiment, we prepared the 2 X RT master mixes using the kit components before preparing the reaction plate. The RT master mix was prepared on ice, adding 2 μl of 10X RT buffer, 0.8 μl of 25X dNTP mix (100 mM), 2 μl of 10X RT random primers, 1 μl of MultiScribe™ Reverse Transcriptase, 1 μl of RNase Inhibitor, 3.2 μl of nuclease-free water, and 10 μl of RNA sample. cDNA PCR was performed at 25˚C for 10 min, 37˚C for 120 min, and 85˚C for 5 min.

The TaqMan Gene Expression Master Mix (Applied Biosystems) was used for the RT-PCR procedure. The RT-PCR reaction mix was prepared using 10 μl of 2X TaqMan Gene Expression Master Mix, 1 μl of 20X TaqMan Gene Expression Assay, and 8 μl cDNA template and water. RT-PCR was performed at 50˚C for 2 min, 95˚C for 10 min, 95˚C for 15 s, and 60˚C for 1 min. Tumor necrosis factor-α (*TNFα*) (Rn01525859_g1) and interleukin-6 (*IL6*) (Rn01410330_m1) expression is reported relative to that of β-actin (Rn00667869_m1). RT-PCR gene expression analysis was performed using the Applied Biosystems StepOne Plus RT-PCR protocol.

Determination of serum alanine transaminase (ALT) level. The serum ALT level was analyzed at 24-48 h to assess liver cell function using a HITACHI-917 auto analyzer and a commercial kit for Human samples (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden Germany).

Histological evaluation. The liver tissue samples were fixed in 10% neutral formalin for histological analysis. These tissues were routinely processed and embedded in paraffin. Sections 5-6 μm thick were cut and stained with Harris Hematoxylin and Eosin (H&E). Tissue samples from all experimental groups were compared.

Statistical analysis. The data were analyzed using analysis of variance (ANOVA) and the Mann-Whitney *U*-test with SPSS version 12.0 for Windows (SPSS, Chicago, IL, USA).

Results and Discussion

After 48 h, a significant difference ($p<0.001$) in serum ALT level was found among the treated groups. In the PH geraniol-treated groups (group VIII and IX), the ALT levels were significantly decreased relative to PH saline- treated (group IV and V) and PH silymarin-treated groups (group VI and VII). In the PH silymarin-treated groups (24-48 h), that were positive control groups, ALT levels were similarly decreased relative to PH saline treated groups (group IV and V) ($p<0.05$) (Figure 1A).

TNFα expression increased within the first 24 h in the saline, Silymarin, and geraniol-treated PHx groups. The *TNFα* level in the PH geraniol-treated groups (group VIII and IX) significantly increased in the first 24 h compared to the control (group I) and sham-operated control (group II and III) groups and 48 h after PH, the level of expression remained the same. A statistically significant difference ($p<0.05$) was found at 24 and 48 h among all groups, except group VII (PH silymarin-treated 48 h), group VIII (PH geraniol-treated 24 h), and group IX (PH geraniol-treated 48 h) (Figure 1B).

IL6 levels in the PH geraniol-treated groups (group VIII and IX) at the 48th h were increased. While a significant difference was not observed in groups VII (PH silymarin-treated 48 h), VIII (PH geraniol- treated 24 h), and IX (PH geraniol- treated 48 h), a statistically significant difference was observed among the other groups (Figure 1C).

Histological analysis showed that liver lobule structure was normal in both control (group I) and sham-operated control (group II and III) groups. In contrast, vacuolization, bleeding, and expansion in the sinusoidal spaces were observed in the PH saline- treated groups (group IV and V). (Figure 2B). In the PH geraniol-treated groups (group VIII and IX), these features were reduced at 24 and 48 h after PH (Figure 2F). Lastly, mitotic activity and vacuolization were observed in PH silymarin-treated (group VI and VII) (Figure 2D) and PH geraniol-treated (group VIII and IX) groups (Figure 2F). Both these groups (Figure 2C and E) and PH saline-treated groups (group IV and V) had tissue integrity (Figure 2A).

Our study investigated the effects of geraniol on regeneration in liver tissue. The results show that geraniol induced a regenerative response after PH. We also found that geraniol triggers *TNFα* and *IL6* expression after PH.

Serum ALT levels were measured to assess liver damage. ALT levels in geraniol and silymarin-treated groups were significantly reduced 48 h after PH compared to the PH saline-treated groups. These results suggest that hepatocytes had functionally recovered from injury. Miura *et al*. found that in rats that underwent 70% PH, ALT levels peaked in the first 12 h and had approached the level of ALT found in controls by 48 h (19). Greif *et al*. revealed that 48 h after the application of erythropoietin, ALT levels decreased, also approaching levels observed in a control group

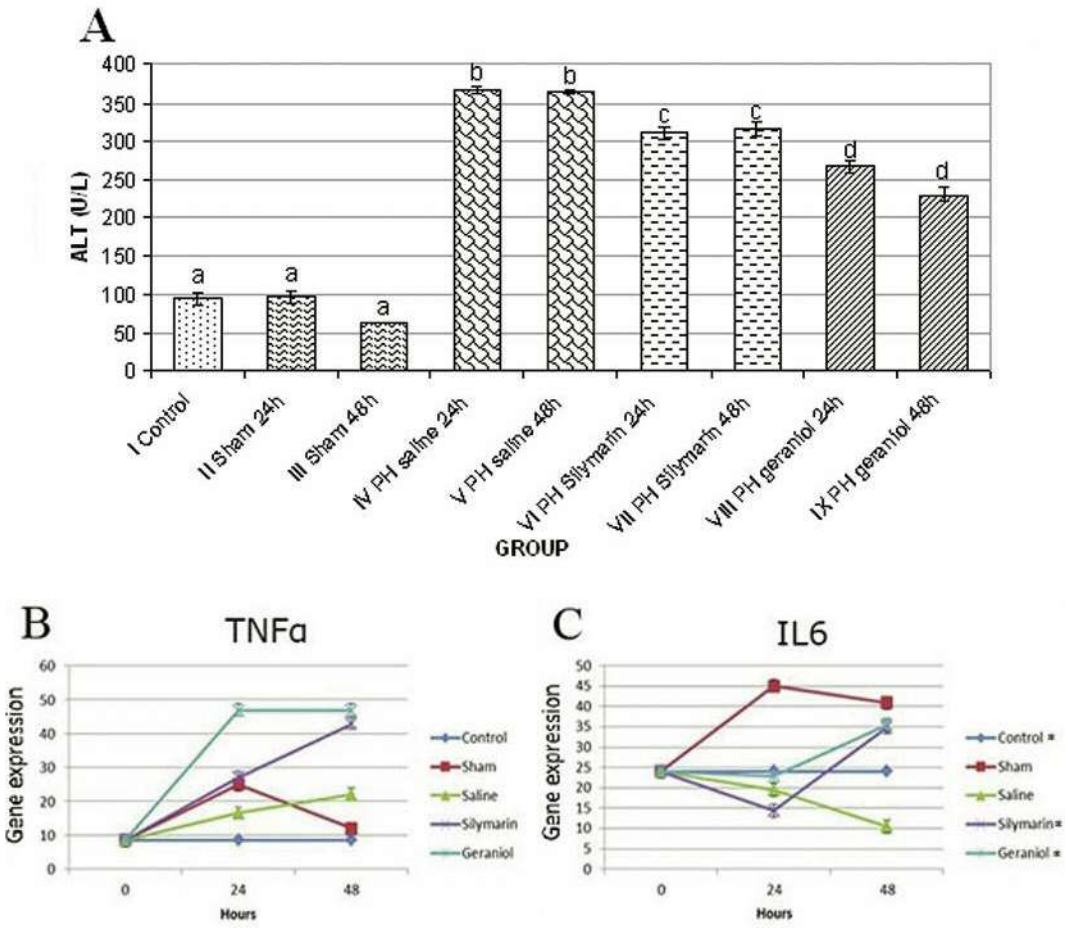


Figure 1. *A: Serum alanine transaminase (ALT) in the different treatment groups. Each value is the mean±SD of 10 animals per group. Bars with the same letters do not differ significantly. Relative gene expression oftumor necrosis factor-α (TNFα) (B) and interleukin 6 (IL6) (C) for all groups. Data are the mean±SD. PH: Partial hepatectomy.*

(20). Within minutes after liver cell loss, hepatocytes enter the cell cycle. Hepatocyte regeneration occurs in developmental stages. In cell cycle TNFα and IL6, triggers transition from G_0 to G_1 (21, 22).

In our study, mRNA expression of *TNFα* and *IL6* were elevated 24 h after PH, and by 48 h the level of gene expression peaked in PH silymarin-treated (group VI and VII) PH geraniol-treated groups (group VIII and IX). In the geraniol-treated groups, TNFα and IL6 expression was similar to those of the PH silymarin-treated groups. Iwai *et al*. showed that 24 h following 67% PH in rats, TNFα, and IL6 levels increased, and their levels peaked 48 h afterwards (8). TNFα and IL6 levels were similar to the control group at 72 h after PH. Scotte *et al*. showed that serum IL6 levels peaked within the first 24 h after PH, then fell and returned to baseline levels (6). Uyanoglu, *et al*. showed that the serum levels of IL6 72 h after PH were similar to levels observed in controls (10). Other studies have shown that the level of IL6 increased at 2 and 4 h after PH. Moreover, others report that IL6 expression remains stable up 48 h after PH, after which it decreases significantly (23-25).

Geraniol applied after PH can increase mitotic activity in hepatocytes, thereby triggering a regenerative process. Studies using PH have found vacuolization, mitotic activity, and remnants of regenerated nuclei (10, 20). We observed an increase in mitotic activity in all groups after PH.

Conclusion

Our study demonstrates the effects of geraniol on liver regeneration in vivo following PH in rats. We found that geraniol induces regeneration in the liver. However, additional research is necessary to reveal the molecular mechanism of this effect and to fully pharmacologically characterize the activity of geraniol using different doses, exposure times, and delivery methods.

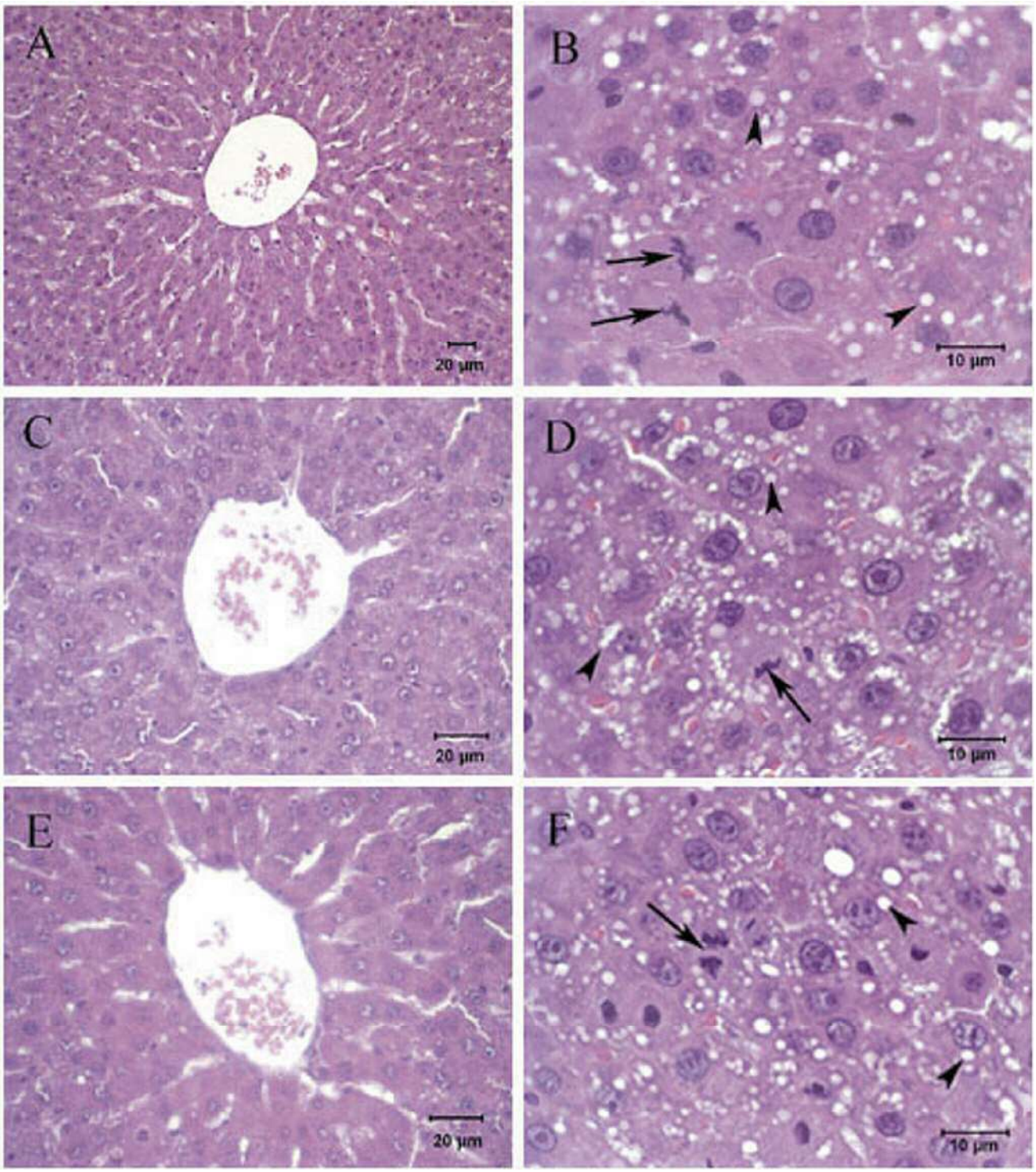


Figure 2. *Hepatocytes in sections of liver stained with hematoxylin and eosin. Tissue integrity was preserved in liver sections of partial hepatectomized (PH) saline-treated groups (group IV and V) (A), PH silymarin-treated groups (group VI and VII) (C) and PH geraniol-treated groups (group VIII and IX) (E). Mitotic figures and vacuolization were observed in PH saline-treated groups (group IV and V) (B), PH silymarin-treated groups (group VI and VII) (D) and PH geraniol-treated groups (group VIII and IX) (F). Mitotic figures are shown with an arrow, and vacuolization is shown with an arrowhead.*

Acknowledgements

This study was supported by the Eskisehir Osmangazi University Research Fund, project number 201119025.

References

1 Ehrenfried JA, Ko TC, Thompson EA and Evers BM: Cell cycle-mediated regulation of hepatic regeneration. Surgery *122*: 927-935, 1997.
2 Palmes D and Spiegel HU: Animal models of liver regeneration. Biomaterials *25*: 1601-1611, 2004.
3 Shimizu T, Togo S, Kumamoto T, Makino H, Morita T, Tanaka K, Kubota T, Ichikawa Y, Nagasima Y, Okazaki Y, Hayashizaki Y and Shimada H: Gene expression during liver regeneration after partial hepatectomy in mice lacking type 1 tumor necrosis factor receptor. J Surg Res *152*: 178-188, 2009.
4 Taub R: Liver regeneration: From myth to mechanism. Mol Cell Biol *5*: 836-847, 2004.
5 Black D, Lyman S, Heider TR and Behrns KE: Moleclar and cellular features of hepatic regeneration. J Surg Res *117*: 306-315, 2004.
6 Scotte M, Masson S, Lyoumi S, Hiron M, Teniere PP, Lebreton JP and Daveau M: Cytokine gene expression in liver following minor or major hepatectomy in rat. Cytokine *9(11)*: 859-867, 1997.
7 Akerman P, Cote P, Yang SQ, McClain C, Nelson S and Bagby GJ: Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. Am J Physiol *263*: G579-585, 1992.
8 Iwai M, Cui T-X, Kitamura H, Saito M and Shimazu T: Increased secretion of tumor necrosis factor and interleukin 6 from isolated, perfused liver of rats after partial hepatectomy. Cytokine *13(1)*: 60-64, 2001.
9 Kapan M, Ipek T, Sad A, Goksel S and Sirin F: Effects of cyclosporin and somatostatin on liver regeneration after partial hepatectomy in rats. Eur Surg Res *28(4)*: 262-269, 1996.
10 Uyanoglu M, Canbek M, Aral E and Baser K: Effects of carvacrol upon the liver of rats undergoing partial hepatectomy. Phytomedicine *15(3)*: 226-229, 2008.
11 Banthorpe DV: Biosynthesis of geraniol and nerol and their p-d-glucosides in pelargonium graveolens and rosa dilecta biochem. Biochem J *130*: 1045-1054, 1972.
12 Chen W and Viljoen AM: Geraniol – a review of a commercially important fragrance material. South African Journal of Botany *76(4)*: 643-651, 2010.
13 de Cássia da Silveira e Sá R, Andrade LN and de Sousa DP: A review on anti-inflammatory activity of monoterpenes. Molecules *18*: 1227-1254, 2013.
14 Cardozo MT, de Conti A, Ong TP, Scolastici C, Purgatto E, Horst MA, Bassoli BK and Moreno FS: Chemopreventive effects of b-ionone and geraniol during rat hepateocarcinogenesis promotion: Distinct actions on cell proliferation, apoptosis, hmgcoa reductase, and rhoa. J Nutr Biochem *22*: 130-135, 2011.
15 Chagas CEA, Vieira A, Ong TP and Moreno FS: Farnesol inhibits cell proliferation and induces apoptosis after partial hepatectomy in rats. Acta Cirúrgica Brasileira *24(5)*: 377-382, 2009.
16 Lapczynski A, Bhatia SP, Foxenberg RJ, Letizia CS and Api AM: Fragrance material review on geraniol. Food Chem Toxicol *46(11)*: 160-170, 2008.
17 Mansour HH, Hafez HF and Fahmy NM: Silymarin modulates cisplatin-induced oxidative stress and hepatotoxicity in rats. J Biochem Mol Biol *39(6)*: 656-661, 2006.
18 Higgins GM and Anderson RM: Experimental pathology of liver. I. Restoration of liver white rat following partial surgical removal. Arch Pathol *12*: 186-202, 1931.
19 Miura Y, Washizawa N, Urita Y, Imai T and Kaneko H: Evaluation of remnant liver function using 13c-breath test in a rat model of 70% partial hepatectomy. Hepato-Gastroenterology *59(114)*: 1-22, 2012.
20 Greif F, Ben-Ari Z, Taya R, Pappo O, Kurtzwald E, Cheporko Y, Ravid A and Hochhauser E: Dual effect of erythropoietin on liver protection and regeneration after subtotal hepatectomy in rats. Liver Transplant *16*: 631-638, 2010.
21 Sakamoto T, Liu Z, Murase N, Ezure T, Yokomuro S, Poli V and Demetris JA: Mitosis and apoptosis in the liver of interleukin-6-deficient mice after partial hepatectomy. Hepatology *29(2)*: 403-411, 1999.
22 Zimmermann A: Regulation of liver regeneration. Nephrol Dial Transplant *19(4)*: 6-10, 2004.
23 Blindenbacher A, Wang X, Langer I, Savino R, Terracciano L and Heim MH: Interleukin 6 is important for survival after partial hepatectomy in mice hepatology. Hepatology *38(3)*: 674-682, 2003.
24 Fukuhara Y, Hirasawa A, Li X-K, Kawasaki M, Fujino M, Funeshima N, Katsuma S, Shijima S, Yamada M, Okuyama T, Suzuki S and Tsujimoto G: Gene expression profile in the regenerating rat liver after partial hepatectomy. J Hepotol *38*: 784-792, 2003.
25 Sudo K, Yamada Y, Saito K, Shimizu S, Ohashi H, Kato T, Moriwaki H, Ito H and Seishima M: Tnf-a and il-6 signals from the bone marrow derived cells are necessary for normal murine liver regeneration. Biochim Biophys Acta *1782(11)*: 671-679, 2008.

Received December 7, 2016
Revised January 31, 2017
Accepted February 7, 2017