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EXPERIMENTAL STUDY

Effect of carvacrol on IL-6/STAT3 pathway after partial hepatectomy in rat liver

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ABSTRACT

BACKGROUND: Carvacrol is a component in the essential oil of Lamiaceae family. Beside its antihepatotoxic and hepatoprotective properties it is known to be a contribution to liver regeneration.

OBJECTIVES: This study aimed to investigate the effects of carvacrol in rat liver regeneration after 70 % partial hepatectomy (PHx) through interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) and mitogen-activated protein kinases (MAPK) pathways.

METHODS: In our study six groups were formed from 3-month old *Wistar albino* male rats. Group I and II were Sham operated; Group III and IV received 1 mL 0.9 % NaCl; and Group V and VI were the groups that were treated with 73 mg/kg carvacrol. Group III, IV, V, and VI animals underwent 68–70 % PHx. Dissection was performed 24 and 48 after surgical procedure. Serum Alanine transaminase (ALT) levels were determined. The liver regeneration rate (RR) was calculated. Histopathological analysis was performed by Hematoxylin&Eosin staining and the proliferation by proliferating cell nuclear antigen (PCNA) immunohistochemical staining in liver samples. In the liver, certain genes expressions in IL-6/STAT3 and MAPK pathways with RT-PCR analysis and protein expression with Western blot analysis were measured.

CONCLUSION: Carvacrol showed a positive effect on hepatocyte proliferation and liver regeneration 24 and 48 hours after PHx *(Tab. 1, Fig. 7, Ref. 39)*. Text in PDF *www.elis.sk.*

KEY WORDS: liver regeneration, IL-6/STAT3 pathway, SOCS3, MAPK pathway, PCNA index.

Introduction

Use of natural substances has increased in recent years to provide a faster healing of liver damaged from various reasons. Carvacrol is a phenolic monoterpene which is abundant in essential oils of lamiaceae family (1). It has antioxidant, antihepatotoxic, and hepatoprotective properties (2). Besides, it was reported to have positive impact on liver regeneration (3).

IL-6/STAT3 is an important signaling pathway for the initiation of regeneration after partial hepatectomy (PHx), when the level of tumor necrosis factor-alpha (TNF-α) and IL-6 rises in the liver (4). TNF-α induces the expression of nuclear factor kappa B (NF-kB) in neighboring cells (5). Activated NF-kB increases release of IL-6. Binding of IL-6 to its receptor leads to cytoplasmic phosphorylation of Janus kinases and STAT3, respectively. The latter enters into the nucleus after dimerization and binds to DNA. Thus, proliferation of hepatocytes begins (5, 6). IL-6 signaling in hepatocytes is modulated by STAT3-mediated stimulation of suppressor of cytokine signaling 3 (SOCS3) (5).

Another critical pathway for liver regeneration and hepatocyte survival is the MAPK pathway, which is initiated by activation of Ras. Ras activation is terminated by hydrolysis of GTP. Activated Ras proteins bind to Raf kinases and cause their embedding to the cell membrane and activation. Raf induces phosphorylation of MAPK/ERK kinase (MEK), which in turn induces phosphorylation of extracellular signal-regulated kinases (ERK). Activated ERK phosphorylates other protein kinases and transcription factors in the nucleus (1) and cytoplasm. Entry of the activated ERK into the nucleus and subsequent phosphorylation of Elk stimulate transcription of immediate early genes (7). Considering all of thesethis, our study aimed to investigate the effect of carvacrol on the rat liver regeneration stimulated by PHx through IL-6/STAT-3 and MAPK pathways.

Materials and methods

Experimental design

Our study was performed after being approved by Local Ethics Committee of School of Medicine in Eskisehir Osmangazi University (approval No: 335/2013). Three-months-old male *Wistar albino* rats, weighing 250 ± 30 g in average were used in the experiment. A total of six groups after randomly selecting six rats into each group were created, as follows:

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Sham: These rats were only exposed to surgical stress without PHx and dissections were performed after 24 (Group I) and 48 hours (Group II).

SP+PHx: These rats were administered intraperitoneally (i.p.) 1mL of NaCl 0.9 % (SP) one hour prior to PHx and dissections were performed after 24 (Group III) and 48 hours (Group IV).

Carvacrol+PHx: These rats received 73 mg/kg carvacrol i.p. one hour prior to PHx and dissections were performed after 24 (Group V) and 48 hours (Group VI).

Carvacrol with 99 % purity was acquired via vapor distillation from *Origanum onites L.* by Prof. KHC Baser (8).

Anesthesia of the rats was established by intramuscular 10 mg/kg xylazine and 70 mg/kg ketamine. PHx was performed according to the technique described by Higgins and Anderson (9).

Histological analysis

Serum ALT levels were determined by HITACHI-917 auto-analyzed with use of Human commercial kits (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden Germany).

Regeneration rate (RR) of the liver samples was calculated by the equation of Child et al. (10) and then chemical fixation was performed with 10 % neutral formaldehyde. Sections 5 µm thick were obtained from all liver tissue samples. Standard H&E staining and immunohistochemistry with commercial PCNA antibodies (abcam ab18197 anti-PCNA) were applied on these histological sections. PCNA index, which was expressed as the percentage of the number of PCNA positive nucleated cells within 1000 parenchymal liver cells that were randomly selected from different section fields, was calculated.

Real Time PCR (RT-PCR) Analysis

Liver tissue samples were isolated by TRIzol (Ambion, Cat. No: 15596-026) with the use of PureLink RNA Mini Kit (Ambion, Cat. No: 12183018A). Obtained RNAs were measured by NanoDrop ND_1000 Spectrophotometer device. RNA samples were prepared with the use of High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems, Cat. No: 4387406) to synthesize cDNA which was required for RT-PCR.

RT-PCR was performed via TaqMan Gene Expression Master Mix (Applied Biosystems, Cat. No: 1108124). Genetic expressions were evaluated based on β-Actin. Brand of primers used in RT-PCR was Life Technologies. The product codes of the genes used were as following: β-Actin: Rn00667869_m1, NFκB p65:Rn01399583_m1, IL-6:Rn01410330_m1, STAT3:Rn00562562_m1, SOCS3:Rn00585674_s1, c-Myc:Rn01519412_g1, RAF-1:Rn00466507_m1, MEK1:Rn01444760_m1, MEK2:Rn00590971_m1, ERK1:Rn00820922_g1, MAPK4:Rn01523937_m1, Elk1:Rn01756649_g1, Ets1:Rn00561167_m1.

Western Blot Analysis

Proteins were isolated using RIPA Buffer (sc-24948) kit. Total amount of protein measured with The Qubit 2.0 Fluorometer Quantitates device was equalized to 60 µg/µL. Western Blot procedure was performed with Novex Chemiluminescent Substrates (WP20005) kit. Primary antibodies were used after aqueous dilution by 1:500. Primary antibodies (Santa Cruz Biotechnology, Inc.) connected with the IL-6/SATA3 pathway included: β-Actin (C4): sc-47778, NFκB p65 (C-20): sc-372, IL-6 (M-19): sc-1265, STAT3 (C-20): sc-482, and SOCS3 (H-103): sc-9023. Primary antibodies (Bioss Antibodies) connected with the MAPK pathway included: c-RAF/RAF-1: bs-1703R, MEK1: bs-1433R, MEK2: bs-0223R, ERK1: bs-1020R, ERK1+ERK2: bs-0022R, Elk1: bs-1398R, c-Myc: bs-4963R, and Ets1: bs-1285R. Secondary antibodies (Santa Cruz Biotechnology, Inc.) were used after aqueous dilution by 1:10000. Secondary antibodies used were Donkey anti-goat IgG-HRP: sc-2020, Goat anti-rabbit IgG-HRP: sc-2004, and Goat anti-mouse IgG-HRP: sc-2005.

Statistical analysis

All statistical analyses were performed through IBM SPSS Statistics Version 20.0 pack software. ANOVA was used to evaluate the differences between groups in terms of serum ALT levels, hepatic regeneration rates, PCNA indices, genetic transcription levels of IL-6/STAT3 and MAPK pathways. Tukey's and LSD tests were used to compare the study groups.

Results

There was a temporal decrease in serum ALT levels between groups. ALT levels were lower in the Carvacrol+PHx groups (Group V and VI) than in the SP+PHx groups (Group III and IV). The statistically significant difference observed between SP+PHx and Carvacrol+PHx groups at 24 hours ($p < 0.01$) was even more increased at 48 hours ($p < 0.001$) (Tab. 1).

Regeneration rare (RR) was detected in the liver at 24 hours and 48th hours of PHx. It was observed that the RR increased with time in the SP+PHx and Carvacrol+PHx groups and this increase was greater in the groups receiving carvacrol. There was no statistically significant difference between SP+PHx and

Tab. 1. Serum ALT levels, liver RR and PCNA index for all experimental groups (Mean ± SD).

Groups			ALT (U/L)	RR (%)	PCNA
Sham	I	24 h	119.83±20.68	---	0.03±0.01
	II	48 h	90.83±10.32	---	0.06±0.04
SP+PHx	III	24 h	370.50±40.86	13.58±1.84	0.22±0.09
	IV	48 h	309.67±13.82+	23.84±2.46+	0.49±0.09+
Carvacrol+PHx	V	24 h	295.17±53.69*	17.39±1.53	0.41±0.08**
	VI	48 h	199.00±60.4**,+	32.57±2.84**,+	0.84±0.06**,+

According to SP+PHx group * $p < 0.01$, ** $p < 0.001$ and + $p < 0.001$ Time dependent statistical differences

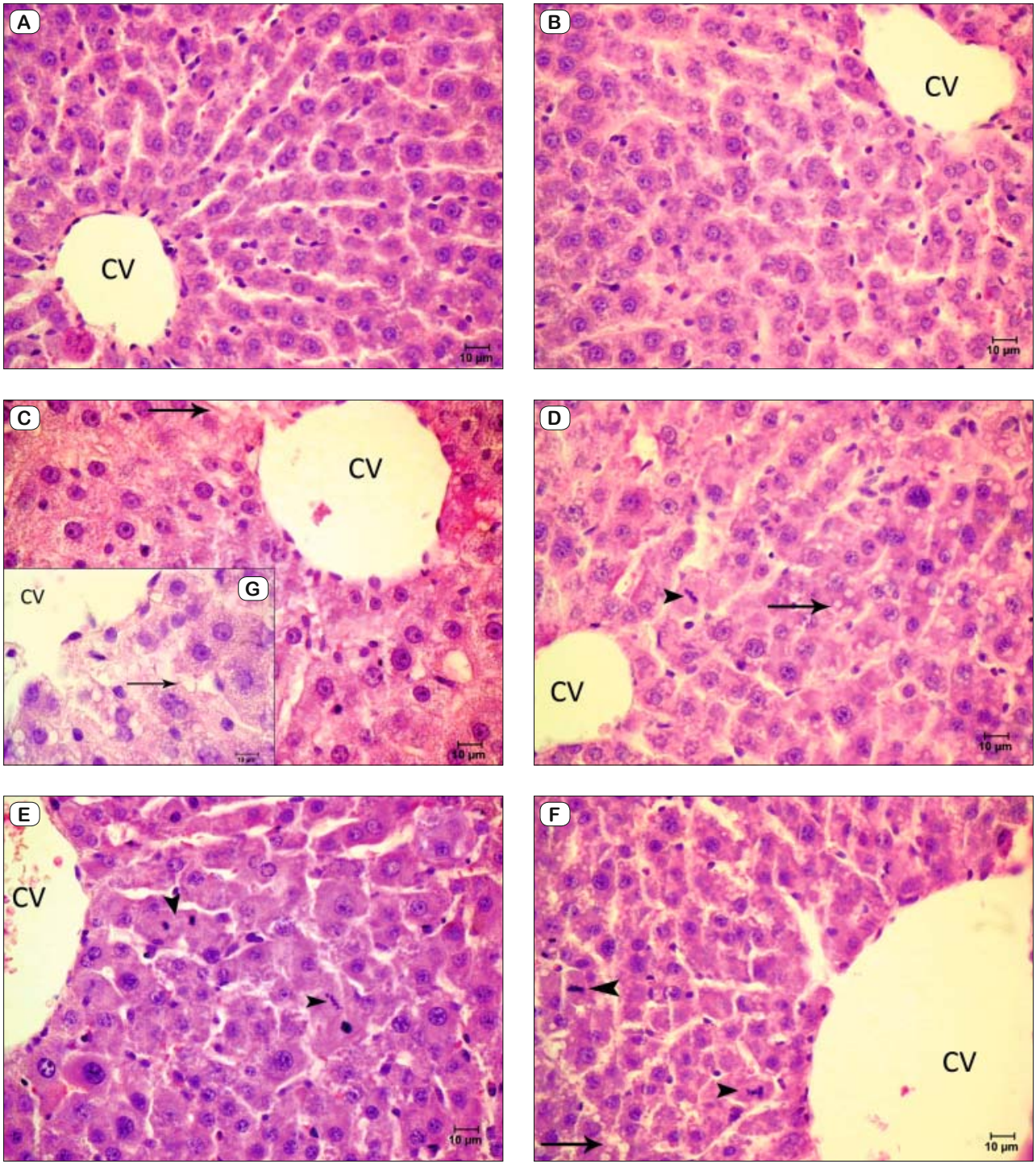

Fig. 1. Liver sections of all experimental group animals (H&E). (A; Group I, B; Group II, C and G; Group III, D; Group IV, E; Group V, F; Group VI). (arrow; vacuolization, arrowhead; mitotic figures, CV; Central vein).

Carvacrol+PHx groups at 24 hours, but significant difference at 48 hours ($p < 0.001$) (Tab. 1).

Histopathological evaluations of liver sections of the Sham group (Group I and II) revealed normal hepatocyte and tissue structure (Fig. 1 A and B). Liver sections in the SP+PHx group (Group III and IV) showed preservation of typical hepatic lobule structure despite the presence of an intensive hepatocyte vacuolization (Fig. 1 C, G and D). Mitotic figures were detected in hepatocytes of Group IV (Fig. 1.D). In Groups V and VI which received carvacrol, it was found that typical liver lobule structure

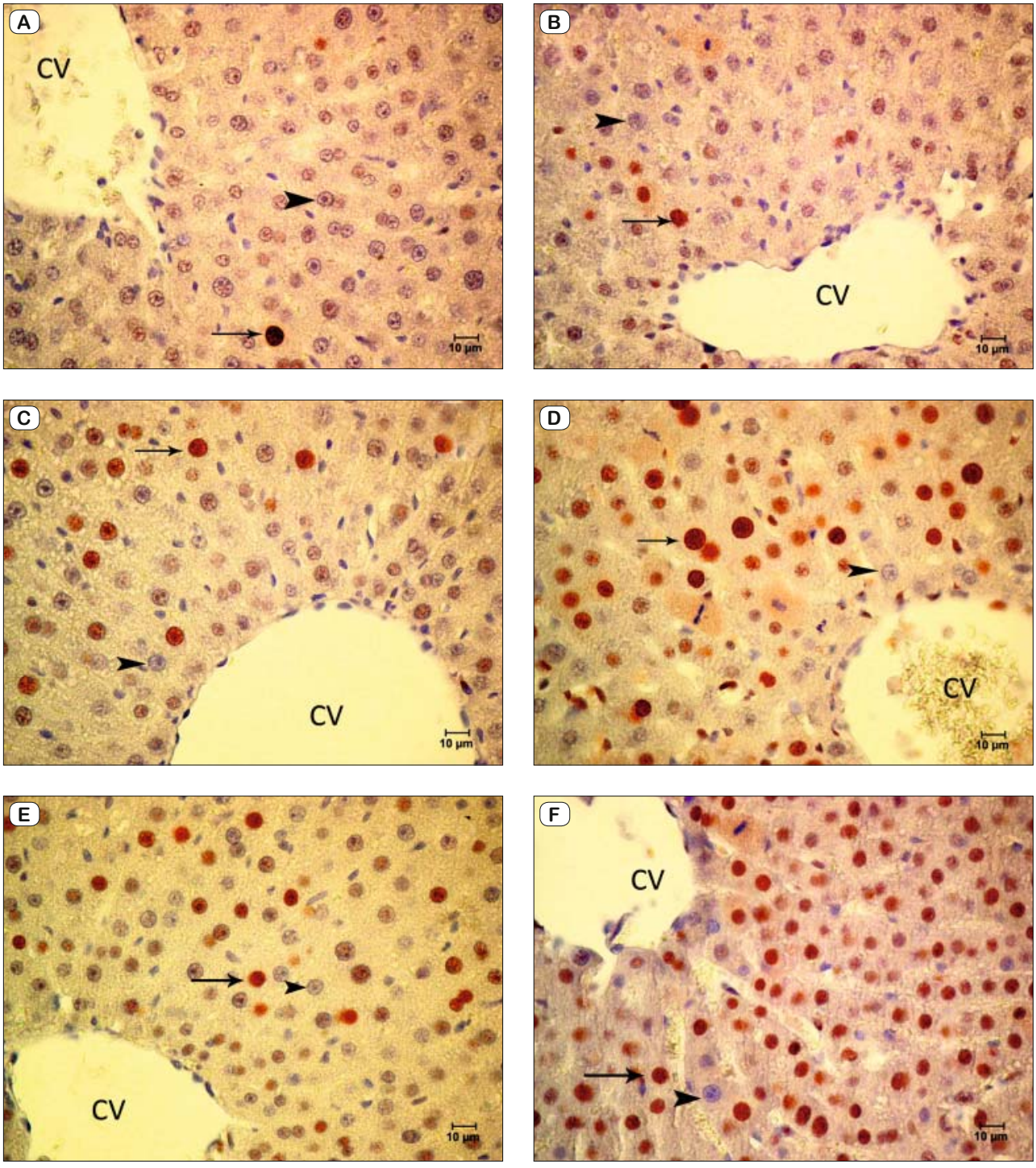

Fig. 2. PCNA positive (arrow) and negative (arrowhead) hepatocytes in liver sections of all experimental group animals. (A; Group I, B; Group II, C; Group III, D; Group IV, E; Group V, F; Group VI).

was preserved, vacuolization was relatively low, and mitotic figures were increased (Fig. 1 E and F).

PCNA assays of liver sections were performed by light microscopy. In Group III (Fig. 2 C) and Group IV (Fig. 2 D) who underwent SP+PHx, the number of PCNA positive cells were detected to raise compared to that in the Sham groups, i.e. Group I (Fig. 2 A) and Group II (Fig. 2 B). It was observed that the number of PCNA positive cells in Carvacrol-treated animals, i.e. Group V (Fig. 2 E) and Group VI (Fig. 2 F) was even higher. There was statistically significant difference between the SP+PHx and

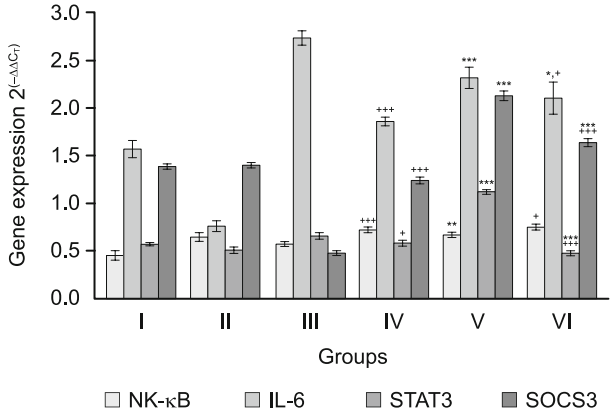

Fig. 3. Expression of genes (NF-κB, IL-6, STAT3, SOCS3) belonging to IL-6/STAT3 pathway (Mean ± SD). * p < 0.05, ** p < 0.01 and *** p < 0.001 According to SP+PHx groups (Group III and IV) statistical differences. + p < 0.05 and +++ p < 0.001 According to time statistical differences.

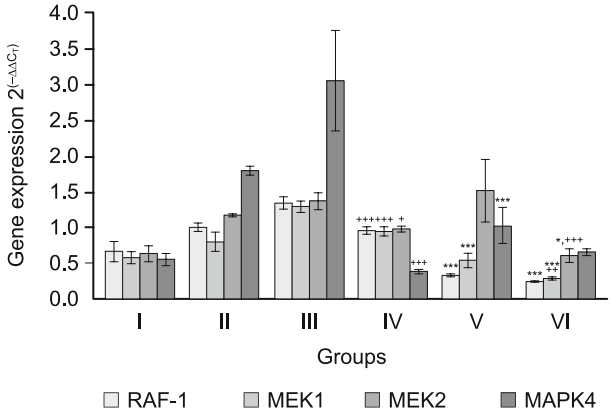

Fig. 5. Expression of genes (RAF-1, MEK1, MEK2, MAPK4) belonging to MAPK pathway (Mean ± SD). * p < 0.05 and *** p < 0.001 According to SP+PHx groups (Group III and IV) statistical differences. + p < 0.05, ++ p < 0.01 and +++ p < 0.001 According to time statistical differences.

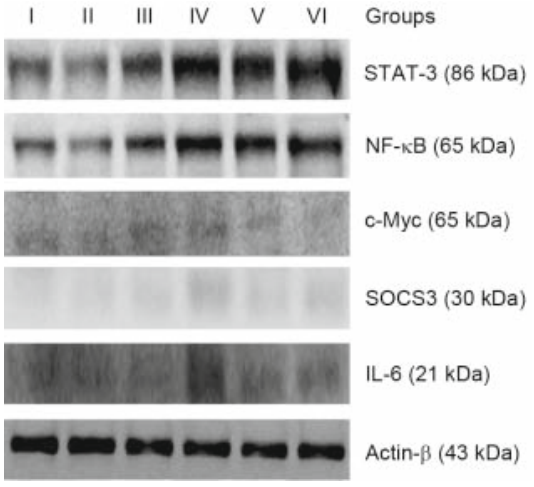

Fig. 4. Band images obtained from Western blot analyzes of protein expressions of IL-6/STAT3 pathway.

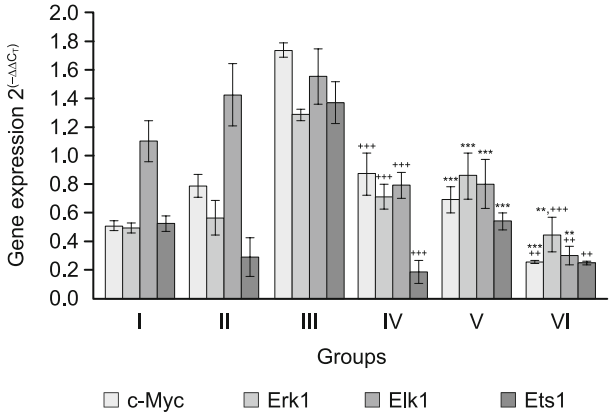

Fig. 6. Expressions of c-Myc, Erk1, Elk1 and Ets1 genes belonging to experimental groups (Mean ± SD). ** p < 0.01 and *** p < 0.001 According to SP+PHx groups (Group III and IV) statistical differences. ++ p < 0.01 and +++ p < 0.001 According to time statistical differences.

Carvacrol+PHx groups at Hour 24 and 48 in terms of PCNA index ($p < 0.001$) (Tab. 1).

NF-κB gene expression was overall higher in Carvacrol+PHx groups than in SP+PHx groups depending on time (Fig. 3). Genetic expression in Group III significantly differed from those in Group IV ($p < 0.001$). While there was a statistically significant difference ($p < 0.01$) in NF-κB expression at 24 hours among the groups, but there was no difference at 48 hours. A statistically significant difference was observed in terms of NF-κB expression in Carvacrol+PHx groups depending on time ($p < 0.05$).

Western blot analysis showed that lower expression of the NF-κB protein from IL-6/STAT3 pathway at 24 hours increased in all groups at 48 hours. NF-κB protein expression was higher in SP+PHx groups than in Carvacrol+PHx groups (Fig. 4).

The expression of IL-6 gene was found to decrease with time in all groups, and more reduced in Carvacrol+PHx groups compared to that in SP+PHx groups (Fig. 3). IL-6 expression in Group III significantly differed from Group IV ($p < 0.001$). While there was a statistically significant difference in IL-6 expression at 24 hours between the groups ($p < 0.001$) and at 48 hours ($p < 0.05$). A statistically significant difference was observed in terms of IL-6 expression in Carvacrol+PHx groups depending on time ($P<0.05$).

Western blot analysis showed that the expression of IL-6 protein, which was low at 24 hours in all experimental groups, increased over time. Compared to that in SP+PHx groups, IL-6 protein expression was higher at 24 hours and lower at 48 hours in Carvacrol+PHx groups (Fig. 4).

STAT3 gene expression was found to decrease over time in all groups. While genetic expression of STAT3 was higher in Carvacrol+PHx groups, it was higher in the SP+PHx groups than in the Carvacrol+PHx groups at 48 hours (Fig. 3). The difference between Carvacrol+PHx and SP+PHx groups in terms of STAT3

gene expressions at 24 hours and 48 hours was statistically significant ($p < 0.001$).

STAT3 protein expression was observed to increase with time in all experimental groups (Fig. 4).

SOCS3 gene expression was higher in the Carvacrol+PHx groups than in the other groups. The expression of SOCS3 gene was increased in SP+PHx groups and decreased in Carvacrol+PHx groups depending on time (Fig. 3) and were statistically different from each other in all PHx groups ($p < 0.001$).

SOCS3 protein expression temporally increased in all groups and this increase was found to be higher in Carvacrol+PHx groups than in SP+PHx groups (Fig. 4).

The expression of c-Myc gene was found to decrease in SP+PHx and Carvacrol+PHx groups compared to Sham groups in a time-dependent manner. Reduction of expression significantly differed between Group V and Group VI, both of which were Carvacrol+PHx groups ($p < 0.01$). Genetic expression of c-Myc was higher in SP+PHx groups compared to that in Carvacrol+PHx group (Fig. 6). It was determined that Group III showed statistically significant difference ($p < 0.001$) in terms of c-Myc gene expression compared to the other groups at 24 hours. At 48 hours, the reduction difference of c-Myc gene expression ($p < 0.001$) found in Group VI was statistically significant when compared with Group IV.

c-Myc protein expression decreased with time in all groups and was the lowest in the Carvacrol+PHx groups (Fig. 4).

The expression of RAF-1 gene, which was high in the SP+PHx and Carvacrol+PHx groups at 24 hours, was detected to decrease after 48 hours (Fig. 5). The difference between Group III and V was found to be statistically significant for RAF-1 gene expression ($p < 0.001$). There was no statistical difference in Carvacrol+PHx groups.

A time-dependent increase in RAF-1 protein expression, one of MAPK pathway proteins, was observed, which were lower in the Sham and SP+PHx groups at 24 hours. On the contrary, elevated expression of RAF-1 protein at 24 hours in Carvacrol+PHx groups decreased at 48 hours (Fig. 7).

The expression of the MEK1 gene, which was high at 24 hours, was found to decrease in all PHx groups at the end of 48 hours (Fig. 5). SP+PHx groups significantly differed from other groups ($p < 0.001$) in MEK1 gene expression at 24 hours. There was a highly significant difference between SP+PHx and Carvacrol+PHx groups in the MEK1 gene expression at 48 hours ($p < 0.001$). There was also highly significant difference in Carvacrol+PHx groups in a time-dependent manner ($p < 0.01$).

The expression of MEK2 gene was found to decrease with time in the SP+PHx and Carvacrol+PHx groups (Fig. 5). At 48 hours, the expression of MEK2 gene was significantly different between Group IV and VI ($p < 0.05$).

It was determined that the expression levels of MEK1 and MEK2 protein, which were high in Sham and Carvacrol+PHx groups at 24 hours, decreased at the end of hour 48 whereas they were low in the SP+PHx group at 24 hours, and increased at 48 hours. Both MEK protein expressions were detected to diminish in both SP+PHx and Carvacrol+PHx groups in a time-dependent manner (Fig. 7).

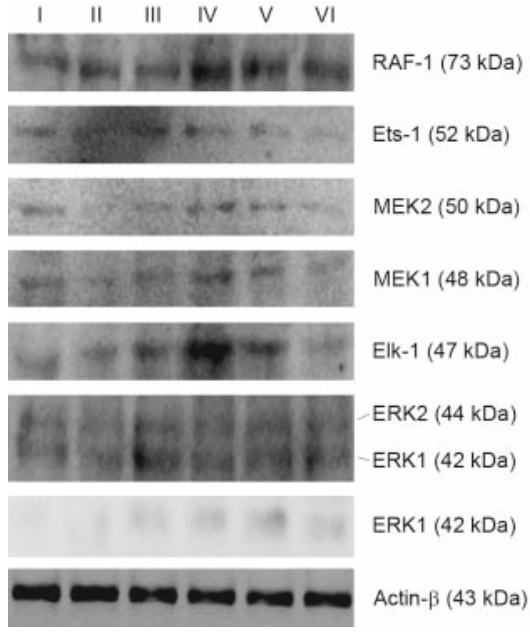

Fig. 7. Band images obtained from Western blot analyzes of protein expressions of MAPK pathway.

In the SP+PHx and Carvacrol+PHx groups, MAPK4 gene expression was high at 24 hours; and decreased with time (Fig. 5). The expression of MAPK4 gene in SP+PHx group showed significant difference ($p < 0.001$) from all other groups at 24 hours. No time-dependent difference was detected in Carvacrol+PHx groups. (MAPK4 protein data were not shown)

In the SP+PHx and Carvacrol+PHx groups, a time-dependent decrease in ERK1 gene expression was observed (Fig. 6). It was found that the expression of ERK1 gene at 24 hours showed significant difference from all groups compared to SP+PHx groups ($p < 0.001$). A statistically significant time-dependent difference ($p < 0.001$) was also found between Group V and VI in terms of ERK1 gene expression. There was statistically significant difference ($p < 0.01$) between SP+PHx and Carvacrol+PHx groups at 48 hours.

ERK1 and ERK1+ERK2 protein expression decreased in all experimental groups depending on time. ERK1 protein expression was higher in the Carvacrol+PHx groups compared to that in the SP+PHx groups. On the other hand, ERK2 protein expression was lower in Carvacrol+PHx groups than in SP+PHx groups at 24 hours; which became elevated at 48 hours (Fig. 7).

It was found that genetic expression of Elk1 time-dependently decreased in SP+PHx and Carvacrol+PHx groups. Elk1 gene expression was detected to be higher in the SP+PHx groups than in the Carvacrol+PHx groups (Fig. 6). At 24 hours, the difference between the SP+PHx and Carvacrol+PHx groups was statistically significant ($p < 0.001$) for the Elk1 gene expression. A time-dependent statistical difference ($p < 0.01$) was also observed to be significant between Carvacrol+PHx groups.

Elk1 protein expression decreased with time in Sham and Carvacrol+PHx groups, but increased in SP+PHx groups. In ad-

dition, Elk1 protein expression was higher in the Carvacrol+PHx groups compared to that in the SP+PHx groups (Fig. 7).

In all experimental groups, Ets1 gene expression, which was high at 24 hours, decreased in hour 48. Ets1 gene expression in Carvacrol+PHx groups was found to decrease in a time-dependent manner ($p < 0.01$), (Fig. 6). After 48 hours, SP+PHx and Carvacrol+PHx groups were found similar in terms of Ets1 gene expression.

In Sham and Carvacrol+PHx groups, expression of Ets1 protein declined with time but increased in SP+PHx groups. Ets1 protein expression was also higher in the Carvacrol+PHx groups than in the SP+PHx groups (Fig. 7).

Discussion

The findings of our study are similar in terms of serum ALT levels and regeneration rate when compared with previous studies. Miura et al (11) reported that in the rats undergoing 70 % PHx, ALT levels reached the highest level during first 12 hours, and then decreased with time, reaching the levels similar to that of control rats at 48 hours. Comparable studies from the literature reported higher serum ALT levels in the PHx-treated groups compared to the Sham group (12) with a decline after treatment (3, 13, 14). Previous studies showed the rate of liver regeneration to usually and time-dependently increase in groups where PHx was performed (15, 16). Uyanoglu et al (3) reported that the rate of regeneration in carvacrol group among those undergoing 70 % PHx was higher than that of those who did not receive carvacrol. In another similar study, it was reported that the regeneration rate of the group treated with erythropoietin 30 minutes before 70 % PHx exhibited more elevation than the group treated with PHx alone (15).

The PCNA index was also investigated in our study because it might support regeneration findings. Consistent with our findings, Gultekin et al (17) found PCNA positive cells in hepatocytes of Sham group animals at 24 hours and 48 hours. The time-dependent elevation of PCNA index was also supported by various studies (16, 18, 19). Yao et al (14) reported that the number of PCNA positive cells increased in this group, as well as the decline of the vacuolization formations observed in hepatocytes 48 hours after PHx, consistent with our results. Several studies also underlined histopathological evidence of vacuolization secondary to PHx (15).

Consistent with our study, it was reported that the NF-κB expression began within 30 minutes (20, 21) and continued until the 14th day after PHx (21), and that NF-κB activation increased 1 to 6 hours and 24 to 36 hours after 2/3 PHx (22). Yang et al (23) investigated NF-κB expression at different time-points in rat livers which had either ligated or non-ligated portal branch and reported that NF-κB expression peaked at 48th hour in all groups and decreased to normal level at Day 7.

IL-6 expression was reported to increase 1 hour after PHx (18, 24, 25). Hosoya et al (26) reported that there was a time-dependent reduction in the expression of IL-6 from 6th to 24th hour. In our study, IL-6 level was similar to the literature in PHx+Carvacrol groups. The expression of post-PHx IL-6 in mice was reported to increase in the first 24 hours by Ogiso et al (27) to decline at 48 hours by Kremer et al (19) and continue to decrease at the end of 72 hours by Lonati et al (28) and Li et al (16).

Post-PHx STAT3 expression was reported to increase time-dependently up to 24 hours, whereas pSTAT3 to decline with time (26). Some investigators reported that post-PHx STAT3 phosphorylation was high at 8 hours (25, 28), and low at 24 hours (28). Ogiso et al (27) advocated that the STAT3 expression after 70 % PHx peaked at 24th hour and reached at trough levels at the end of 48th hour. The findings of our study appeared to be consistent with the results of previous studies. Interestingly, the STAT3 gene expression in the Group VI was decreased at 48 hours in our study. This is because although supported by the literature, the reduction of the STAT3 expression at 48 hours compared to that at 24 hours was not expected. Therefore, the fact that the IL-6 signal in hepatocytes was regulated by IL-6/STAT3-mediated stimulation of SOCS3 (5) necessitated discussion of our whole study, taking SOCS3 data into consideration.

Several studies reported that the SOCS3 expression started to increase after PHx, reaching maximal levels after 6 hours and decreasing with time (22). In mice, activation of SOCS3 was reported to be high at 24 hours after 2/3 PHx but to decline at 48 hours (6, 19, 28). Similarly, SOCS3 expression increased in the first 24 hours and decreased with time in PHx+Carvacrol groups of our study.

Consistent with our study, it was reported that the c-Myc RNA expression level, which was highest at 24th hour based on RT-PCR analysis, became declined at 48th hour after 70 % PHx (29). On the contrary, some studies reported increased c-Myc RNA expression at 48 hours after liver transplantation in rats (30). Chang et al (31) reported that c-Myc gene expression peaked at 30 hours, then declined and rose again at 72 hours after 2/3 PHx, as measured by microarray analysis of hepatic samples.

Considering genetic expressions of NF-κB, IL-6, STAT3, SOCS3, and c-Myc in our study with the data from the literature together, the dependence of these five factors to each other has been once again revealed. Genetic expressions were detected to increase at 24 hours in our study groups, i.e. Sham (Group I), SP+PHx (Group III), and PHx+Carvacrol (Group V) groups, respectively. Similar findings were also found for SOCS3 gene expression. Nevertheless, 48-hour data showed that the increase in SOCS3 gene expression was remarkable in Group VI to suppress STAT3, which we believed to complete its task. The time-dependent decline in SOCS3 gene expression in the PHx+Carvacrol groups of our study was also seen for IL-6, STAT3, and c-Myc gene expressions. On the other hand, in our study increased NF-κB gene expression was exhibited in similar study hours between groups, higher expression by carvacrol compared to SP+PHx could be regarded as a favorable outcome. The continued elevation in NF-κB expression at 48 hours in our study is thought to be an indicator of the positive contribution to the molecular mechanism of hepatic regeneration and healing by carvacrol. This is further supported by the genetic expressions of IL-6, STAT3, and SOCS3.

MAPKs play a role in modulation of hepatocyte function by mechanical and oxidative stress-dependent regulation (32). Therefore, the state of the molecules in the MAPK pathway was also investigated. Consistent with our study, Silverman et al (33)

reported that post-PHx mRNA of c-RAF increased up to 24 hours and decreased in the following period. However, it was also reported that the expression of c-RAF-1 gene measured at 24 and 48 hours after pancreatectomy in rats showed a time-dependent elevation (34). Some authors reported that suppression of MKK4 activated other members of the MAPK family, such as MKK7, thus improving hepatocyte regeneration as Elk1 expression increased (35). The same study also suggested that suppression of MKK4 led to increased p-RAF-1 expression and decreased expressions of RAF-1 and MEK2.

Hamano et al (36) stated that the ratio of p-ERK1/2 to ERK1/2 increased with time after PHx in control group rats and that ERK1/2 phosphorylation was detected at 24 hours after PHx and continued until 48 hours. Pajaud et al (29) found that there was a time-dependent increase in ERK1/2 activation after PHx in wild-type mice. In another study, it was argued that ERK protein expression increased significantly in erythropoietin-treated rats 48 hours after 70 % PHx (15). In our study, expression of ERK1 protein revealed a time-dependent reduction in SP+PHx and Carvacrol+PHx groups. However, expression of ERK1/2 protein decreased with time in SP+PHx groups and increased in Carvacrol+PHx groups.

Several studies reported that Elk gene expression peaked at 24 hours, then declined, and rose again at 72 hours in liver samples after 2/3 PHx, as measured by microarray analysis (31, 37). Our Elk1 findings determined by RT-PCR are supported by the literature.

Terada et al (38) reported that Ets1 mRNA expression increased up to 24 hours after 1-hour of ischemia and 24/48 hours of reperfusion. However, Tanaka et al (39) reported that Ets-1 mRNA and protein expression increased between 6 and 24 hours after 1-hour of ischemia and 24/48 hours of reperfusion. Consistently, Ets1 mRNA expression in the SP+PHx and Carvacrol+PHx groups increased during first 24 hours and then decreased in a time-dependent manner in our study.

In this study where the effects of single intraperitoneal doses of carvacrol on the liver regeneration of rats induced by PHx via IL-6/STAT3 and MAPK pathways were investigated, the regeneration was thought to be positively affected. Apart from our histopathological assessments, this was further supported by genetic and protein expressions of NF-κB, IL-6, STAT3, SOCS3, and c-Myc involved in IL-6/STAT3 pathway. The positive impact of carvacrol on this pathway was also demonstrated on the expression of genes and proteins in the MAPK pathway. In our study, carvacrol was shown to be more effective in the regenerative process through IL-6/ STAT3 pathway. We believe that this regenerative effect of carvacrol may be important in terms of complementary medical practice, which needs to be confirmed by further investigation of the effects at different times and doses of carvacrol on this pathway in the same experimental model.

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