Bcl-2 and Bax Messenger RNA Gene Expression Detected by Reverse Transcriptase Polymerase Chain Reaction after Partial Hepatectomy and Licorice in Rats

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Summary Liver regeneration is a complex physiological response that takes place after the loss of hepatocytes caused by toxic or viral injury or secondary to liver resection. This work purposed to investigate the implication of bcl-2 and bax expression as an important contribute in liver transplant donors and also the possible modulatory effect of licorice on their expression. Forty eight male albino rats weighing 250–320 g were divided into 6 groups (8 animals for each). The rats underwent partial hepatectomy (PH) represents 3 groups (sacrificed after 24, 48 and 72 h after PH) and other 3 groups after oral receiving of licorice extract for 4 weeks (2.4 g/kg b.w.). The resected liver was considered as control (zero time). The expression of bcl-2 and bax mRNA of normal liver and liver of PH were measured by reversed transcriptase polymerase chain reaction (RT-PCR). The distinct changes in the expression of the mRNA encoding these 2 genes were confirmed by an immunohistochemical staining of the liver tissues (streptavidin-biotin method). The analysis of mRNA level revealed a significant increase of anti-apoptotic bcl-2 mRNA expression after 24 h ($p < 0.05$), while, it was declined in the following 2 d. In contrast, bax mRNA expression showed delayed increase reaching a peak at 48 h after PH. The pretreatment with licorice caused a statistically significant increase in bax mRNA expression; meanwhile, the change in bcl-2 expression was not significant when compared to the control. Furthermore, immunohistochemical staining of regenerating liver with bcl-2 and bax antibodies showed that the changes in the bcl-2 and bax protein levels were similar and consistent to that found for mRNA. It was suggested that licorice may provide a mechanism in the induction of apoptosis through modulation of bcl-2 and bax expression in regenerating liver as a model for chemopreventive drug of cancers.

Key words Bcl-2, Bax, mRNA, Hepatectomy, Licorice.

Bcl-2 and bax are 2 discrete members of a gene family involved in the regulation of cellular apoptosis. The bcl-2 family is the best characterized group of apoptosis-mediating factors, which include bcl-2, mcl-1, bcl-x, bax, bak, and several others. Although, its members share close structural homologies, their biologic functions differentiate into apoptosis promoting (bax, bak, bcl-xS) or apoptosis-inhibiting (bcl-2, mcl-1, bcl-xL) properties (Que and Gores 1996, Friess *et al.* 1998, Xu *et al.* 2005).

Although, bcl-2 and bax genes share partial nucleotide sequence homology and their encoded polypeptides have critically conserved amino acid sequences in 2 functional domains, their individual products appear to have opposing functions with regards to their effects on the apoptotic sensitivity of a cell (Craig 1995, Karavias *et al.* 2003, Xu *et al.* 2005).

Bcl-2, a gene located at chromosome 18q21, blocks programmed cell death without affecting cellular proliferation. It was first described in B cell leukemia and later was detected in other malignant tumors. In the liver, bcl-2 presence has been reported in areas of cholangiolar proliferation. In

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addition, bcl-2 expression was present in periportal hepatocytes in liver sections from rats with bile duct ligation, where it is believed that it protects metaplastic hepatocytes from cholestasis-induced apoptotic death (Doglioni *et al.* 1994, Kurosawa *et al.* 1997, Akcali *et al.* 2004).

Glycyrrhiza glabra (licorice) "the grandfather of herbs" originated in the Mediterranean and Middle East and has been used medicinally (Ody 1993). It was one of the most commonly prescribed herbs then, as it is today. Traditional uses include the treatment of peptic ulcers, asthma, pharyngitis, malaria, abdominal pain, and infections. The primary active constituent of *Glycyrrhiza*, as it relates to hepatic disorders, is the triterpene glycoside glycyrrhizin (also known as glycyrrhizic acid or glycyrrhetic acid) (Tyler *et al.* 1988, Dhiman and Chawla 2005).

Glycyrrhizin (GL), an aqueous extract of licorice root is known to have various immunomodulatory and biological response-modifier activities. GL was used in patients with hepatitis to reduce the activity of liver inflammation. However the mechanism underlying the anti-inflammatory activity of GL is poorly understood. Long treatment with GL prevented the development of cancer of the liver. The mechanism by which GL reduces the progression of liver diseases is unknown. Many studies have reported the antihepatotoxic effects of GL (Wang and Han 1993, Haraguchi *et al.* 1998, Gumprecht *et al.* 2005).

Glycyrrhiza exerts antiviral activity *in vitro* toward a number of viruses, including hepatitis A and HIV. Intravenous glycyrrhizin has been shown to be effective against viral hepatitis, in particular chronic viral hepatitis. *Glycyrrhiza* has been shown to stimulate endogenous interferon production in addition to its antioxidant and detoxifying effects (Susuki *et al.* 1984, Baba and Shigeta 1987, Acharya *et al.* 1993, Abe *et al.* 2003).

Liver regeneration is also one of the few physiologic models of growth regulation. So, an understanding of the molecular mechanisms governing liver regeneration may be useful for designing therapies for patients with hepatitis, liver injury, a liver transplant or cancers.. After a two-thirds partial hepatectomy in which the small lobes of the liver are left intact and without injury, the majority of liver cells reenter the cell cycle (Fausto and Mead 1989, Michalopoulos 1990).

Liver regeneration can be initiated in several ways. Classical methods for initiating liver regeneration in animal models involve partial hepatectomy (Michalopoulos and DeFrances 1997). For that, the purpose of this work to investigate the implication of Bcl-2 and bax expression as an important contribute in liver transplant donors and also the possible modulatory effect of licorice on their expression.

Materials and methods

Experimental animals

The study included 48 male albino rats, weighing 250–320 g. The animals were housed in stainless-steel cages, 4 rats per cage, at room temperature (22°C) and 12-h dark/light cycles. They were maintained on standard laboratory diet with tap water *ad libidum* throughout the experiment, except for an overnight fast before surgery. Four weeks before surgery 24 rats had received licorice extract (2.4 g/kg b.w. of crude herb) (Paolini *et al.* 1998). Roots of licorice were cut and sifted, then, 14.4 g were added to 250 ml of boiling water. After the water was poured over the root, it was allowed to simmer for an additional 5 min. It was then cooled and filtered. 10.4 ml was taken by orally gastric tube divided in 2 times per day.

Surgical proced

The surgical procedure was performed under strict sterile conditions, using ether anesthesia. In all animals, a midline incision (4–5 cm in length) was done in the upper abdomen on day zero, and partial hepatectomy (approximately 70%) was performed according to the protocol described by Higgins and Anderson (1931). Eight samples of liver tissue were obtained and were considered as

Table 1. Experimental groups and treatment protocol

Examined groups	Treatment	No. of animals	Sacrificed after 70% PH
Control	—	—	Zero time
Group 1	Partial hepatectomy	8	24 h
Group 2	Partial hepatectomy	8	48 h
Group 3	Partial hepatectomy	8	72 h
Group 4	Treated with licorice for 4 weeks	8	24 h
Group 5	Treated with licorice for 4 weeks	8	48 h
Group 6	Treated with licorice for 4 weeks	8	72 h

Zero time=control group.

control. Subsequently, the abdominal incision was closed. The animals were assigned into 6 groups (each consisting of 8 rats) and were sacrificed at 24 h, 48 h and 72 h after PH was performed Table 1. In all particular time points and immediately before euthanasia, 2 pieces of liver tissue were obtained from each liver sample: 1) A piece was transferred into liquid nitrogen for molecular genetic study (RT-PCR). 2) A piece was fixed in 10% neutral formalin for paraffin section for immunohistochemical examinations.

Sample preparation and RNA isolation

The liver tissues were rinsed in liquid nitrogen, and immersed and stored in -80°C refrigerator for RNA extraction. Total RNA was isolated from frozen liver tissue according to RNA isolation kit (Gentra, Minneapolis, MN 55441 USA). In brief, 3 ml cell lysis solution was added to 50–100 mg ground liver tissue, homogenized quickly in liquid nitrogen by using 5–10 strokes with a tube pestle. 1 ml protein–DNA precipitation solution was added to the cell lysate and placed into ice bath for 10 min. Centrifuged at 15,000 rpm for 5 min. The precipitation proteins and DNA will form a tight pellet (Chomczynski and Sacchi 1987).

The supernatant containing the RNA was poured into a clean 1.5 ml microcentrifuge tube containing 300 μl 100% isopropanol, then centrifuged at 13,000–16,000 rpm for 3 min. The RNA was seen as a small, translucent pellet. 300 μl 70% ethanol was added, centrifuged at 16,000 rpm for 1 min. 50 μl RNA hydration solution was added. RNA was allowed to rehydrate at least 30 min on ice. Purified RNA sample were stored at -70 – -80°C . The purity and integrity of total RNA were monitored by absorbance of ultraviolet spectrophotometer at 260/280 nm, and electrophoresis was carried out on a denaturing formaldehyde agarose gel and stained with ethidium bromide. The yield of extracted RNA was determined by the spectrophotometer at 260 nm where, 1 absorbance unit (A260) equals 40 μg of single standard RNA/ml (Masson *et al.* 2000).

RT-PCR system

300 ng mRNA was reversely transcribed to single-stranded cDNA by power transcriptase at 42°C for 1 h followed by heating for 5 min at 99°C . First, $-$ strand cDNA was synthesized. The resulting single strand cDNA was amplified by PCR, 3' primer (A1) and 5' primer (A2) following parameters: 94°C denaturation step for 1 min, annealing step at 68°C for 6 min and extension step at 72°C at the last cycle.

Primer design and reverse transcription PCR

The primer was designed for bcl-2 and bax by using the c-DNA sequences of rat homologues of these genes.

(Gene Bank accession number of bcl-2s is NM. 016993, and bax is 576511) (Miglio *et al.* 2000). The primer pairs used for each gene were shown in Table 2.

Table 2. Sequence of primers used in RT-PCR experiment

Genes	Primer sequence	bp
β -actin	Fw: 5'-GGCCACAATGGCTGACCATTC Rev: 5'-AAGGTGACAGCATTGCTTC	112
Bcl-2	Fw: 5'-GCAGCTTCTTTCCCCGAAGGA Rev: 5'-AGGTGCAGCTGACTGGACATCT	272
Bax	Fw: 5'-AACTTCAACTGGGGCCGCGTGGTT Rev: 5'-CATCTTCTCCAGATGGTGAGCGAG	372

Miglio *et al.* (2000).

Detection of PCR product by gel electrophoresis

In all the reactions, the mRNA target chosen as control was β -actin gene. PCR amplifications of fragment of β -actin c-DNA were performed as the quantiation step was done using gel documentation system (gel Pro-Analyzer Version 3.1) software Media Cybermetica USA (a software system used for end point quantitation of nucleic acid samples).

The photographed gel was analyzed using the gel document system (gel pro version 0.3) to get the maximum optical density of the bands of the PCR products. A plot was constructed for the input copy number of the standards in relation to the optical density (OD) of the product bands. Using the constructed curve, the input copy number of the sample was anticipated from the OD of its product.

In this study, the results were expressed as a ratio of target mRNA/ β -actin mRNA an external control for each sample.

The initial denaturation step was at 95°C followed by 33 cycles for bcl-2 and 29 cycles for bax for 30 s, annealing for 30 s at 55°C for bcl-2 and 60°C bax, and extension for 30°C seconds at 72°C. The PCR products were electrophoresed on a 2% agarose gel.

Immunohistochemistry

The detection of cells that express bcl-2 and bax proteins relied on immunohistochemistry based on a streptavidin biotin peroxidase method (Biogenex, San Ramon, CA, USA). Briefly, 4 μ m thick sections were dewaxed in xylene and hydrated through graded concentrations of ethanol alcohol. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 15 min. Sections were then processed in a microwave oven twice for 5 min each time at high power, and subsequently stained with polyclonal antibodies to bcl-2 (Santa Cruz, CA, USA at a dilution of 1 : 40), and bax (Santa Cruz, CA, USA, at a dilution of 1 : 1000). All incubations were performed for 30 min at room temperature. Between the steps, sections were washed in TBS. Diaminobenzidine (Sigma Fast DAB tablets, D-4293, Sigma St. Louis, MO, USA) was used as a chromogen. Cytoplasmic staining for bcl-2 and bax was considered as positive. The stain for bcl-2 and bax proteins was recorded as present or absent. For negative control purposes, the same streptavidin-biotin technique was used on tissue sections in which 1% BSA in PBS was substituted for the primary antibody. (Stahelin *et al.* 1999, Salakou *et al.* 2001, Ravazoula *et al.* 2002).

These investigations were performed in Cytogenetic and Molecular Biology Laboratory in Girls' College in collaboration with Laboratories of Molecular Biology in National Cancer Institute, Egypt.

Statistical analysis

The data were expressed as median and range. Statistical analysis was carried out using the T test and correlation analysis significance was defined as $p < 0.05$.

Table 3. The expression of bcl-2 and bax mRNAs in rats partial hepatectomy and after treatment with licorice

Groups	No. of animals	Bax expression	Bcl-2 expression	β -actin. expression
Zero time (control group)	—	0.9	0.5	61.89
After treatment with licorice	—	1.1	0.6	61.89
24 h after PH	8	1.5	1.2*	60.26
After treatment with licorice	8	1.7	1.4	60.26
48 h after PH	8	2.0*	1.0	59.77
After treatment with licorice	8	2.0*	0.7	59.77
72 h after PH	8	0.9	0.6	59.4
After treatment with licorice	8	1.2*	0.6	59.4

PH=Partial hepatectomy. * $p<0.05$ significant.

Fig. 1. Graphical description of bcl-2 mRNA expression after partial hepatectomy with ■ or without □ licorice. **Fig. 2.** Graphical description of bax mRNA expression after partial hepatectomy with ■ or without □ licorice.

Results

Animal survival

All results from groups; zero time, 24, 48 and 72 h after hepatectomy survived the whole experiment and were in good health at the time of euthanasia.

Bcl-2 mRNA expression by RT-PCR

In the normal liver, Bcl-2 mRNA could be detected in the hepatocytes. The expression was significantly upregulated at 24 h after hepatectomy, Table 3. The bcl-2 mRNA level was 1.2. Later on, bcl-2 expression was returned gradually nearly to the control levels in 72 h after PH. It was 0.6 (Fig. 1).

In the rats treated with licorice, the expression of bcl-2 mRNA was slightly increased above each corresponding time point. The expression of bcl-2 at 24 h, 48 h and 72 h (1.4, 0.7 and 0.6 re-

spectively) was not significant when compared to the control group.

Bax mRNA expression by RT-PCR

All results regarding, bax mRNA expression were shown in Table 3. After about 70% partial hepatectomy, the bax mRNA expression was 1.5 at 24 h and increased gradually to the maximum level at 48 h, it was significant when compared to the control group (Fig. 2). Also, in the rats treated with licorice, bax mRNA expression was increased, it was significant when compared to the control and decreased gradually to 1.0 at 72 h (Fig. 2).

Moreover, the quantitative RT-PCR gel electrophoresis was observed to examine the bcl-2 and bax mRNA expression (active products) in rat liver after PH for different times (zero, 24, 48, and 72 h) and after treatment with licorice (Figs. 3a, b, and c). The first lane represents the DNA marker (M) (100–500 bp). Lane (1) represents the control group (zero time), lanes (2–4) represent mRNA expression after PH and lanes (5–7) represent mRNA expression in rats underwent PH after treatment with licorice.

It revealed that, the bands of both bcl-2 and bax mRNA expression ranged between 250 bp to 400 bp. β -actin is a gene encoding a structural protein which, constitutively expressed and its expression was used as normalization control for RNA loading.

Densitometric analysis by gel scanning 24 h after PH and treatment with licorice

It was noted that, the mean band density (bd) of bcl-2 mRNA after PH was 43.7 ± 3.4 . It was significant ($p < 0.05$) when compared to control group (Table 4) and it was slightly increased (37.23 ± 2.9) after treatment with licorice. In the same time, the mean bd of bax mRNA after PH was increased (34.5 ± 1.5). After treatment with licorice bax mRNA was increased to (39.7 ± 2.6). This increase was not

Fig. 3. RT-PCR products from in RNX of partial hepatectomy (PH) rat of liver pretreatment with or without (control) licorice. a) 24 h, b) 48 h and 72 h after PH. Lanes C2–L4, represent control, without pretreatment of licorice, from individual rat, and L5–L7 represent RT-PCR products after PH with pretreatment of licorice.

Table 4. The average band density of control rat liver, PH and after treatment with licorice

Gene expression	Zero time (control group)	Mean bd after 24 h PH	Mean bd after 48 h PH	Mean bd after 72 h PH
Bcl-2	20.3 ± 1.6	$43.7 \pm 3.4^*$	26.3 ± 2.6	22.6 ± 2.5
After treatment with licorice	21.2 ± 1.7	37.23 ± 2.9	28.1 ± 1.4	22.41 ± 1.9
Bax	32.4 ± 2.6	34.5 ± 1.5	$43.5 \pm 2.7^*$	34.6 ± 2.8
After treatment with licorice	35.6 ± 1.8	39.7 ± 2.6	$47.1 \pm 2.3^*$	$38.1 \pm 4.2^*$
β -actin	36.7 ± 2.4	38.9 ± 3.1	38.9 ± 3.2	33.9 ± 4.7
After treatment with licorice	36.7 ± 2.4	38.7 ± 3.2	38.5 ± 2.9	35.78 ± 4.5

* $p < 0.05$ significant. bd=band density, PH=partial hepatectomy.

Fig. 5

Fig. 6

Figs. 4-6. Densitograms of RT-PCR products by mRNA after partial hepatectomy of rat liver with pretreated licorice. Fig. 4, 5 and 6, correspond to the panel a) 24 h, b) 48 h, and c) 72 h of Fig. 3, respectively. Vertical scale: arbitrary density. Numbers in figures correspond to bands in Fig. 3.

Fig. 7. Immunohistochemical analysis of licorice pretreated liver in section after partial hepatectomy (PH). Expression of bcl-2 and bax proteins are presented by slightly dark tone in the liver section. a is control at zero time after PH, b and d represent liver sections at 24 h, 48 h and 72 h after PH, respectively.

significant when compared with the control group (Fig. 4).

48 h after PH and treatment with licorice

It was observed that, the mean band density of bcl-2 mRNA after PH was 26.3 ± 2.6 . It was decreased than 24 h but it was still more than control group. After treatment with licorice, the mean

band density increased to 28.1 ± 1.4 . On the other hand, the mean band density of bax mRNA after PH was (43.5 ± 2.7). It was increased more than 24 h and after treatment with licorice to reach (47.1 ± 2.3). It was significant ($P < 0.05$) when compared to control group (Fig. 5).

72 h after PH and treatment with licorice

It showed that, the mean band density of bcl-2 and bax mRNA expression returned to, nearly, the control group (22.6 ± 2.5). However, the mean band density of bax mRNA was (34.6 ± 2.8). After treatment with licorice the increase (38.1 ± 4.2) was significant when compared to control group (Fig. 6).

From the previous data, it was concluded that, the maximum expression of bcl-2 (anti-apoptotic gene) was after 24 h and decreased gradually to reach the normal level after 72 h. Also, the maximum expression of bax mRNA (pro-apoptotic gene) was after 48 h. The treatment with licorice increased bax expression after 48 h and 72 h after PH.

Immunohistochemistry study

In the normal liver, bcl-2 protein could not be detected in the hepatocytes and ductal epithelial cells (Fig. 7a). At 24 h after PH, bcl-2 was only expressed in cholangiolar proliferating cells (Fig. 7b). Later on 48 h, bcl-2 expression was restricted on cholangiolar epithelial cells (Fig. 7c), but it was lower when compared to that of 24 h. Moreover, bcl-2 protein was expressed in hepatocytes and cholangiocytes at 72 h (Fig. 7d).

Regarding bax protein expression, the normal liver could not be detected in reaction (Fig. 7a). At 24 h after PH bax protein expression was detected in few hepatocytes (Fig. 7b). However, an up-regulation of bax protein was recorded at 48 h after PH (Fig. 7c). Whereas at 72 h bax protein expression was returned to almost normal control levels (Fig. 7d). After treatment with licorice, bax protein was notably increased at 72 h after PH.

Discussion

This study investigates the active production (mRNA expression) by RT-PCR technique and protein presence of antiapoptotic gene (bcl-2) and proapoptotic gene (bax) after PH at 24 h, 48 h, and 72 h and after treatment with licorice.

Regarding the control group before PH, bcl-2 and bax mRNA expression was detected. The obtained data agrees with authors and disagrees with others. Krajewski *et al.* (1994) and Masson *et al.* (2000) who used RT-PCR method reported that bcl-2 and bax mRNA expression was detected in control group. They explained that because bcl-2 seems to be produced exclusively by non-parenchymal cells and especially cholangiocytes, and so, a sensitive method such as RT-PCR is required for detection of hepatic bcl-2. In contrast Tzung *et al.* (1997) reported that in control group the concomitant expression of bcl-2 and bax could not be detected, they did not find specific sites of bcl-2 mRNA production.

On the other hand, after 24 h from PH, the expression of bcl-2 was increased. It was significant when compared to the control group. This observation is in agreement with Xu *et al.* (2005) who confirmed that 41 genes including bcl-2 gene were overexpressed at 24 h after PH. They demonstrated that, the increase in bcl-2 expression at 24 h (intermediate phase) is due to, the hepatocytes synthesize the DNA. The increase of mitochondria glycerol-3phosphate dehydrogenases gene expression at 24 h assumed to provide enough ATP for DNA synthesis. In another opinion, Vail *et al.* (2002) reported that overexpression of Bcl-2 can delay cell cycle progression and DNA replication after 24 h. Other investigators stated that bcl-2 inhibit the apoptosis and contribute to cell survival and resistance of cells against damage. In the same time, increase the rate at which cells withdraw from the cell cycle to become quiescent (Fan *et al.* 1998, Takehara *et al.* 2001).

In contrast, bax which is considered to be a central regulator of apoptosis is a promotor of programmed cell death. However, bax mRNA expression was slightly increased above the basal level. This finding is in agreement with the previous result of Masson *et al.* (2000) who reported that, bax expression was maintained, at 24 h post-PH in the same almost basal levels as before hepatectomy. Other authors Kren *et al.* (1996) and Fan *et al.* (1998) showed that, the alterations in bax expression are essentially in agreement with all studies regarding the presence of the particular oncogene in regenerating livers.

In the same time, some authors revealed that, bax may bind to bcl-2 forming bax/bcl-2 heterodimers, The ratio of bax to bcl-2 determines the susceptibility of a cell to apoptosis. Thus, bax/bcl-2 heterodimers predominate in cells that overexpress bcl-2, and the susceptibility of these cells to apoptosis is reduced (Oltvai *et al.* 1993, Yang and Korsmeyer 1996).

While, after treatment with licorice, bcl-2 levels was slightly insignificantly increased. The data of the present work suggested that, this increase may be due to, the stimulatory and the anti-oxidative mechanisms of licorice which protected against apoptosis. This finding is in agreement with recent studies, (Gumprecht *et al.* 2005, Dhiman and Chawla 2005) who demonstrated that, licorice is a potent inhibitor of the mitochondrial permeability transition, and reactive oxygen species generation. So that, licorice exhibits proapoptotic properties, and a potent inhibitor of apoptosis and necrosis in a manner consistent with its antioxidative effect.

In addition bcl-2 mRNA expression at 48 h after PH was reduced than before. However, Bax mRNA level was increased to the maximum level. It was significant when compared with control group. This data is coincides with Masson *et al.* (2000) and Xu *et al.* (2005) who decided that, in the late phase of liver regeneration (48 h after PH), seven genes including bax gene, were induced to express at 48 h after PH.

Previous reports had shown that bax protein is a homologue of bcl-2 that promotes apoptosis. Kren *et al.* (1996) and Gordon *et al.* (2000) described that, increased level of bax protein are localized to the mitochondria of rat livers after PH during the same time that cytochrome c is released. This observation suggests that hepatocytes are removed after PH *via* apoptotic pathway dependent on relative levels and localization of bax protein, thus decided that the apoptosis associated with bcl-2 and bax is modulated at the transcript and protein level during liver regeneration (LR) suggesting a role of these genes products in normal levels of growth.

On the other hand, Sakamoto *et al.* (1999) and Guo *et al.* (2000) concluded that bcl-2 and bax genes can increase the rate of apoptosis in hepatocellular carcinoma (HCC) by reducing their expression or changing the ratio with other genes. This suggests that regenerative response may predominate resulting in restoration of hepatic mass, and apoptosis happens later in order to destruct the excess hepatocytes.

The present data suggested that bax is the main regulator of tissue homeostasis. It contributes to the elimination of damaged cells in normal tissues and balances the appropriate cell number under the circumstances of physiologic cell proliferation and tissue repair.

The presently reported data showed that, after treatment with licorice the bax mRNA level was significantly increased after 48 h from PH more than those without licorice administration. Similarly, result were explained by Pan *et al.* (2003), indicated that licorice extract and structurally related substances have a positive effect on the cell viability, DNA fragmentation and capase activity, it was suggested that licorice induced apoptosis is triggered by upregulation of bax. In addition, the induction of apoptosis by licorice may provide a pivotal mechanism for its cancer chemopreventive action.

In the terminal phase of LR (72 h after PH), bcl-2 and bax mRNA expression was returned to the normal basal level. The present data is supported by other investigators who reported that the reduction in the expression of proapoptotic (bax). It was found that 19 genes were induced in 72 h after PH. It might be related to enhanced effect of some growth factors in liver proliferation to pre-

vent apoptosis and insure survival of the tissues and the cause of the reduction in the expression of anti-apoptotic (bcl-2) family of genes has been unclear (Xu *et al.* 2005). On the other side, after treatment with licorice, the bax mRNA expression was increased to a significant level. This is similar to Dhiman and Chawla (2005) who proved that licorice improves the subacute liver failure and improves liver functions in patients with hepatic failure, chronic hepatitis and cirrhosis. It also, reduces the mortality among patients with cirrhosis and reduces the development of liver cancer in patients with virus C.

Finally, from all above mentioned findings, the present study concluded that the early bcl-2 increase (after 24 h) may be responsible for rapid improved liver regeneration. On the other hand, up-regulation of bax (after 48 h) may be responsible for the increased rate of apoptosis. It is speculated that, one of the mechanisms could be the suppression of apoptosis as a result of increased bcl-2 expression, and suppressed bax expression.

While, the licorice stimulated the expression profile of bax family members during liver regeneration. It was therefore, recommended that licorice is used as chemopreventive drug of cancers. Further studies examining the possible beneficial or modulator roles of licorice in hepatic disorders, including achievable tissue concentrations, mode of administration, metabolism and potential toxicity is recommended.

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