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ISSN: 2637-4501

Annals of Gastroenterology and the Digestive System

Open Access | Research Article

Traditional Chinese Medicines Maintain the Regenerating Cirrhotic Rat Liver Model After Partial Hepatectomy

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Received: Dec 18, 2020

Accepted: Feb 08, 2021

Published Online: Feb 12, 2021

Journal: Annals of Gastroenterology and the Digestive System

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Partial hepatectomy; Liver regeneration; Traditional Chinese medicines; Thioacetamide; Cell cycle; Cirrhotic liver regeneration.

Abbreviations: HGF: Hepatocyte Growth Factor; PHx: Partial Hepatectomy; IGF-I: Insulin-Like Growth Factor I; CP: Codonopsis Pilosula; BK: Bupleurum Kasi; ESL: Elephantopus Scaber L.; SMB: Salvia Miltorrhiza Bunge; Sm: Silymarin; FAK: Focal Adhesion Kinase; TAA: Thioacetamide.

Abstract

Background: Liver exposed to toxic injury have exhibited to regenerate through a compensatory growth process. The aim of this study was to establish the hepatoprotective properties of Elephantopus scaber L. in cirrhotic rat regeneration partial hepatectomy.

Methods: Cirrhosis was induced by Thioacetamide (TAA, 200 mg/kg) administration for 6, 24, 72 to 168 h, then received surgical 70% cirrhotic PHx was examined. Rats were sacrificed after 6, 24, 72 and 168 h regeneration. Rats were fed 25mg/kg traditional Chinese medicines including Codonopsis Pilosula (CP), Salvia Miltiorrhiza Bunge (SMB), Bupleurum Kasi (BK), Elephantopus Scaber L. (ESL) and Silymarin (Sm), for all process. The cirrhotic remnant liver mass and regeneration weight were measured. The cirrhotic remnant and regeneration liver were detected by western blotting and RT-PCR analysis.

Results: Results showed BK was induced HGF and FAK protein expression levels at 72 and 168 h cirrhotic remnant increased ($P<0.01$). Sm is a positive control. Cyclin D1 was enhanced by BK and SMB at 72 and 168 h, however, Cyclin E was increased at 72 h cirrhotic remnant by ESL, but at 168 h was by ESL and BK. TAA at 6 and 24 h was no effects, without discussions. Cirrhotic remnant mass was decreased at 72 h ($P<0.01$), but liver regeneration weight was increased after 24 h PHx ($P<0.05$). Cirrhotic regeneration PHx, ESL was maintained protein expression of HGF, IGF-I and Cyclin D1/pRb increases at 24 and 72 h PHx by western blotting analysis. From RT-PCR results showed, BK was enhanced Cyclin E/E2F mRNA expression at 24 h PHx cirrhotic regeneration by RT-PCR analysis, but ESL was at 72 h. Bcl 2 protein and mRNA at 24 and 72 h PHx cirrhotic regeneration increased by ESL induced. In contrast, Bad and Cytochrome c.

Conclusions: ESL protect effects on TAA-induced cirrhotic rat liver after PHx.

Cite this article: Tzeng YM, Jia-Ping W. Traditional Chinese Medicines Maintain the Regenerating Cirrhotic Rat Liver Model After Partial Hepatectomy. Ann Gastroenterol Dig Syst. 2021; 4(1): 1031.

Introduction

Liver regeneration is a compensatory growth process when liver went on toxic injury. On the other words, liver regeneration is a maintaining liver functions homeostasis physiological response. Cirrhotic stage is the terminal liver diseases process, then maybe induce tumor growth [1]. However, during cancer stage also need partial hepatectomy to therapy patients. Partial tumor growth is the important stage to stop cancer cell transfer [2]. The regeneration cirrhotic after partial hepatectomy is the important subject in this stage, especially, in the long term complementary supplements. In recent, traditional Chinese medical herbs maybe can help liver protective to return a non-proliferative state. Over-proliferation cell lead to uncontrolled growth induced tumor cell growth [3]. This process results in cell cycle delayed in G1 phase [4]. TGF-β1 is a biological important molecule to control hepatocytes cell cycle induced proliferation. After the regeneration cirrhotic partial hepatectomy the liver then returns to a non-proliferative stage [5]. There is little known about the cirrhotic rat liver regeneration after partial hepatectomy maintains liver mass and growth how to protective liver functions. During liver regeneration, traditional Chinese herbal plays an important role. Because liver damage, the native hepatocyte proliferation function can't maintain integrated whole liver function [6]. Chinese herbal medicines have been used to treat liver disorders for thousands of years in the East. For several years, they have become a promising therapy internationally for pathological liver conditions to have hepatoprotective effects [7]. Elephantopus Scaber L. (ESL) has hepatoprotective effects in a folk medicine of Taiwan plants. The structure of scabertopin, isoscabertopin, deoxyelephantopin, isodeoxyelepantopin, diphroelephantopin and elascaberin was elucidated isolated on the whole Elephantopus scaber L. plants [8]. Deoxyelephantopin has been found exerted anticancer effects on various cancer cells from cell cycle arrest [9]. Codonopsis Pilosula (CP) is a widely Chinese herb in China that has long been used to treat liver disease reported to stimulate cell survival signals [10], be due to have a variety of immunomodulatory activities [11], inhibit inflammatory cytokines, and reduce scar formation. It has long been used for treating liver disease in China. Salvia Miltorrhiza Bunge (SMB) is a Chinese herbal, the main active component of Salvianic acide A. Salvianic acide A could scavenge free radicals, reduce mitochondrial membrane permeability transition in rat liver mitochondrial [12]. The other component isolated from Salvia miltorrhiza Bunge is Tanshinone II-A, which protective effect may through breaking the chain reaction of superoxidation to decrease their cytotoxicity by scavenging lipid free radicals, and be considered to promote blood flow and alleviate blood stasis [13]. Bupleurum Kasi (BK) is an important traditional Chinese crude drug for treating hepatitis malaria to exhibit various biological activities. For example, anti-inflammatory, anticancer, hepatoprotective and immunomodulatory effects have demonstrated in rats after oral administration. Silymarin (Sm) is as a well-known hepatoprotective agent from Silybum marianum seeds as a cytoprotectant for the treatment of liver disease. Silymarin is currently the world's most effective treatment for liver disease. It has shown positive effects in treating nearly every known form of liver disease including cirrhosis, hepatitis, necrosis and liver damage due to drug and alcohol abuse [14]. Silymarin (Sm) is a liver protection drug for different liver diseases. It is with minor side effects, good safety profile and most importantly at an affordable price. It has been investigated for use as an anticarcinogen, and a supportive treatment for liver damage. In vitro and animal studies have suggested that milk thistles active ingredient, silymarin, promotes hepatocyte proliferation and survival [15]. Recently, in present study aim to establish the effective hepatoprotective property of Chinese herbal medicines in cirrhotic rat liver regeneration after partial hepatectomy. Mimic the patient with hepatic tumor in the body with the goal of preventing arresting metastatic cancers. To determine whether Chinese herbal medicines protected liver in cirrhotic rat liver regeneration after partial hepatectomy to ensure that the partial remnant was able to maintain sufficient function. Chinese herbal medicines have been applied to hepatic growth liver regeneration in partial hepatectomy [16]. PHx is a delayed the cell cycle response and, in particular, prolongs the time from the G1 to S phase. In addition, extensive remodeling of the hepatic occurs shortly after PHx. PHx is believed to play a primary role in liver regeneration to promote cell proliferation, and morphogenesis through regulated DNA synthesis [17]. These factors regulate the cell cycle in different ways such as TGF-β1, HGF and IGF-I regulation. Liver regeneration has presumably evolved to protect the wild animals from the catastrophic liver loss caused by toxins or tissue injury. TGF-β1, HGF and IGF-I regulate this process by providing both stimulatory and inhibitory signals for cell proliferation. HGF and IGF-I stimulate the hepatocyte cell cycle. Almost immediately after PHx application major changes occur in the complete mitogen expression for hepatocytes and in the expression of a relatively cell cycle large number of genes [18]. TGF-β1 is a start cell cycle protein beginning G0 phase, in PHx, TGF-β1 acting through its signaling pathway to stop the cell cycle at the G1 phase to stop proliferation, induce differentiation, or promote apoptosis. TGF-β1 is a very special protein during liver regeneration control hepatocytes when to start and when to stop regeneration.

Methods

Experimental

Male rats weight 180–220 g was obtained from the Animal House of National Science Council in Taiwan. Rats were housed to a cage in a room with a controlled temperature of 22 ± 5°C, relative humidity of about 60, free access to standard food in pellets and tap water. Acclimatized for 1 week prior for beginning of experiments. All of the rats were orally administered the drug Thioacetamide (TAA) which was purchased from Sigma Aldrich and dissolved in sterile saline three times (200mg TAA/Kg) for 6, 24, 72 h and 7 consecutive days (168 h). Rats that ingested thioacetamide exhibited induced cirrhotic rat liver tissues. 70% PHx was then administered for regeneration as discussed below (Figure 1).

Experimental the cirrhosis rats partial hepatectomy (PHx)

The cirrhotic rats were subjected to 70% PHx. At different time point after hepatectomy, the livers were collected. After injecting ketamine subcutaneously at a dose of 30 mg/kg, liver resections consisting of 70% of the cirrhosis liver mass were performed in the partial hepatectomy group. All surgical operations were performed the same as PHx. The cirrhotic liver tissues were obtained during operations at 6, 24, 72 and 168 h PHx. All operations were performed between 8:00 AM and 12:00 PM to minimize diurnal effects. After procedure completion the rats were placed under a lamp to prevent hypotermy and then placed into cages (five animals per cage) with a continuous supply of food and water. Rats were sacrificed at 6, 24, 72 and 168 h after the operation by cervical dislocation. The postoperative regenerating cirrhosis livers were excised and

washed in PBS, then immediately frozen in liquid nitrogen. The animal group in which no surgery was performed, was used as the corresponding sham group with the time noted as "0" in the quantitated graphs.

Hot-water extracts prepared from Chinese herbal medicines

The hot-water extract was prepared by boiling the dried roots with distilled water for 1 h. The extract was filtered, freeze-dried and keep at 4°c. The extraction yield from Codonopsis Pilosula (CP) was 41.34%, Salvia Miltiorrhiza Bunge (SMB) was 56.95%, Bupleurum Kaoi (BK) was 43.24%, Elephantopus Scaber L. (ESL) was 31.84%, Silymarin (Sm) was 25 mg/kg. The dried extract was dissolved in distilled water use for the whole experimental process.

Western blot analysis

Proteins were separated using 12% SDS-PAGE and then transferred to nitrocellulose. Membranes were blocked in 5% milk (diluted in Tris-buffered saline and 0.1% Tween 20) and incubated with the appropriate primary antibodies (HGF, FAK, cyclin D1, cyclin E, pRb, IGF-I, Bcl 2, Bad, cytochrome c, TGF-β1 and α-tubulin) at 4 °C overnight and HRP anti-IgG was used as secondary reagent. After extensive washing, the targeted proteins were detected using Enhanced Chemiluminescence (ECL).

Reverse transcriptase-polymerase chain reaction

Total RNA (0.5 ug) was derived from liver plus primers by Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR). The first-strand synthesis kit was applied according to the manufacturer's instructions, the PCR primers included Cyclin D1, Cyclin E, pRb, E2F, Bcl-2, Bad, TGF-β1, cytochrome c and GAPDH (Table 1). The RT-PCR results were analyzed based on the assessment of product amounts in ethidium bromide agarose gel electrophoresis. The initial denaturation step was at 95°C, then at annealing temperature and extension at 72°C. The final extension at 72°C for 10 min was applied to all the reactions and the PCR products were electrophoresed on 1.0% agarose gel.

Quantification of Western Blot and RT-PCR

The intensity (area x density) of the individual bands on western blots was measured by densitometry. The background was subtracted from the calculated area. The area density of the individual bands on Western blot and RT-PCR were measured by densitometry.

Statistical analysis

All data examined were expressed as mean ± SEM (n=6). For Western blot and analysis, quantitation was carried out by scanning and analyzing the intensity of the hybridization signals using FUJIFILM Imagine program. Statistical analysis of the data was performed using SigmaStat software. Comparison between the control or sham group was made using the two -way ANOVA test. A p value of less than 0.05 and 0.01 were considered to be statistically significant.

Results

Bupleurum kaoi (BK) induced HGF, FAK and Cyclin D1 protein expression increases in G1 Phase at 72 and 168 h cirrhotic animal model with thioacetamide, but Elephantopus scaber L. (ESL) in S Phase.

We detected the role of traditional Chinese medicine in the toxic injury process in rats. The HGF and FAK protein expression levels at 72 and 168 h after TAA treatment (Figure 1B and 1C) found that traditional Chinese medicines, Bupleurum Kaoi (BK) and Silymarin (Sm) improved HGF and FAK protein expression levels after TAA toxicity injury ($P<0.05$). Silymarin (Sm) is a positive control to protect liver functions. The Cyclin D1 and Cyclin E protein expression levels at 6, 24, 72 and 168 h after TAA were detected by Western blot (Figure 2A and 2B). According to the results, we sure Cyclin D1 and Cyclin E declined, when after TAA oral administration 72 and 168 h ($P<0.05$). However, early 6 and 24 h TAA-injury did not find Cyclin D1 and Cyclin E protein expression levels decreased until 72 to 168 h. However, all traditional Chinese medicines induced Cyclin D1 and Cyclin E expression increases at 6 h TAA-induced injury. After 24 h TAA-induced injury, TAA still did not affect Cyclin D1 and Cyclin E expression levels increases ($p<0.01$). ESL, SMB and Sm induced Cyclin D1 expression levels increased was observed at 24 h ($P<0.01$), but did not find BK-induced increases. ESL, SMB and CP induced Cyclin E protein expression increases after 24 h TAA oral administration. Therefore, we found ESL hold Cyclin D1 and Cyclin E in G1 and S phase. After TAA oral administration 72 and 168 h, we could find Cyclin D1 and Cyclin E protein expression levels decreased. BK induced Cyclin D1 increases during this time, however, we did not find the same results in Cyclin E expression levels at 72 and 168 h TAA-injury induced. However, ESL induced Cyclin E protein exhibited increased expression after 72 and 168 h- TAA treatment ($P<0.05$). ESL lost induced Cyclin D1 expression at 72 and 168 h TAA-induced injury, but maintained at S phase induced Cyclin E expression. On the other hand, BK induced Cyclin D expression increases led cell cycle hold at G1 phase after 72 and 168 h TAA-injury regeneration. From Figure 1 results, we can find BK induced HGF, FAK and Cyclin D1 protein expression levels increased can hold cell cycle at G1 phase after 72 and 168 h TAA-injury regeneration. However, ESL could maintain Cyclin E protein expression increases in cell cycle, S phase, during 6, 24, 72 and 168 h TAA-injury regeneration. Strangely, TAA-injury regeneration at 6 and 24 h did not find Cyclin D1 and Cyclin E decreased, may be TAA toxic induced injury regeneration stronger during this time.

Elephantopus scaber L. still maintained cell cycle G1 phase at 24 and 72 h cirrhotic rat liver regeneration partial hepatectomy.

Commonly after liver injury, the injury site repairs with internal scar tissue as quickly as possible. After toxic injury, liver regeneration and proliferation were induced. Therefore, 70% tissue section partial hepatectomy (PHx) was done in cirrhotic liver tissue after 6, 24, 72 and 168 h. After PHx, remnant liver weight was reduced at 72 h ($p<0.01$), conversely, liver regeneration weight was increased (Figure 3A). Liver regeneration weight was increased at 24 ($P<0.05$), 72 ($P< 0.01$) and 168 h ($P<0.01$) cirrhotic-PHx rats. In the cirrhotic rat liver regeneration PHx, liver regenerating sham stage did not find Cyclin D1 and pRB protein expression increases, but we can find HGF and IGF-I increased. However, hepatocytes cannot fully respond to cirrhotic rat PHx liver regeneration to the growth factors, HGF and IGF-I, stimulated by TAA-injury observed suppressed. HGF and IGF-I protein expression decreased at 24 and 72 h cirrhotic rat liver regeneration PHx using Western blot (Figure 3B). Traditional Chinese medicines maybe could help cell cycle from G1 to S phase to increase DNA synthesis and rebuild the lost hepatic tissue. At 72 h cirrhotic regenerative liver can find significant decreases (Figure 2). Therefore, we examined at 72 h cirrhotic rat liver regeneration PHx only. Sm has no significant effects on Cyclin D1/pRb at 24 h PHx operated cirrhotic regenerative

liver (Figure 3B). However, ESL and Sm still induced extracellular HGF and IGF-1, but only ESL induced Cyclin D1 and pRB protein expression at 24 h PHx after cirrhotic TAA-induced. At cirrhotic TAA-induced liver regeneration operated 72 h PHx, HGF and pRb protein expression increases only by ESL, during this time, we did not find Sm increases. However, ESL and Sm induced Cyclin D1 protein expression increases were observed. That is why we examined only after 72 h cirrhotic TAA-induced liver regeneration rats, because 168 h TAA after PHx was withdrawn. Therefore, we found liver regeneration weight the highest increases. Short-term 6 and 24 h TAA-induced injury did not affect Cyclin D1 and Cyclin E protein expression (Figure 2). ESL may act as a cell cycle progression agent to make primed cells progress. Starting with expression of a large number of immediate growth factors in the regenerating stage, hepatocytes can fully respond to stimulate cell cycle from G1 phase to S phase to increase DNA synthesis and rebuild the lost hepatic tissue. ESL induced Cyclin D1 mRNA expression levels at 24 h cirrhotic rat liver regeneration PHx (Figure 3C). Interestingly, ESL did not enhance Cyclin E/E2F mRNA increases. ESL maintained at G1 phase, not S phase. On the other hand, ESL maintained Cyclin D1 and pRB mRNA expression in G1 phase at 72 h cirrhotic rat liver regeneration PHx. In contrast, ESL did not induce E2F mRNA increased in S phase, which can suggest ESL still hold cell cycle in G1 phase at 72 h cirrhotic rat liver regeneration PHx.

Elephantopus scaber L. (ESL)-induced TGF-β1 activity in cirrhotic rat liver regeneration partial hepatectomy.

Starting with the G0 phase expression of immediate growth factors, TGF-β1, in the regenerating cirrhotic rat liver. Furthermore, we detected detonator protein, TGF-β1, in cell cycle G0 phase protein and mRNA expression level of regeneration liver at 6, 24, 72 and 168 h in cirrhotic rat liver regeneration PHx by western blotting and RT-PCR (Figure 4A and 4B). ESL increased TGF-β1 protein and mRNA expression level at 72 h cirrhotic rat liver regeneration after 6 h PHx ($p<0.05$), no effects at 24 h, decreased at 72 h ($p<0.05$), but then increased at 168 h PHx ($p<0.05$). Sm is a positive control in cirrhotic rat liver regeneration PHx. We suggest that TGF-β1 gene and protein expression level increased at 72 h cirrhotic rat liver regeneration at 6 h PHx started in priming G0 phase, stopped at 24 h and 72 h PHx in G1 and S phase, but increased at 168 h cirrhotic rat liver regeneration after PHx. TGF-β1 regulates cell cycle beginning and terminal at different time.

Elephantopus scaber L. induced Bcl 2 protein expression level and suppressed Bad and cytochrome c at 24 h and 72 h PHx regenerating cirrhotic rat liver.

In the 72 h cirrhotic animal model with TAA and then PHx, we detected anti-apoptosis protein, Bcl2, and apoptosis proteins, Bad and Cytochrome c, in the 72 h regenerating cirrhotic rat liver at 24 and 72 h PHx by western and RT-PCR (Figure 5 and Figure 6). The results showed that ESL induced Bcl-2 protein expression level increased at 24 and 72 h PHx after TAA-induced cirrhotic liver (Figure 5A). Interestingly, almost all traditional Chinese medicines have functions, including TAA-treatment without medicines. That because of TAA toxicity withdrawal after 72 h PHx. Bad protein expression level was decreased at 24 h PHx after 72 h TAA treatment by SMB, ESL and Sm ($P<0.05$) (Figure 5B). Surprisingly, ESL also lost Bad protein effects in the regenerating cirrhotic rat liver at 72 h PHx, but no significant difference. Cytochrome C protein expression level was decreased by CP, ESL and Sm ($p<0.05$) at 24 h PHx after 72 h TAA-induced cirrhotic liver, but no significant difference compared with Sham at 72 h PHx after 72 h TAA-induced cirrhotic liver (Figure 5C). ESL and Sm induced Bcl 2 mRNA expression levels increased at 24 and 72 h PHx in cirrhotic rat liver regeneration partial hepatectomy ($p<0.05$) (Figure 6A). BK, CP, ESL, and Sm induced Bad mRNA expression levels decreased at 24 h and 72 h PHx in cirrhotic rat liver regeneration PHx ($p<0.05$) (Figure 6B). BK, CP, ESL, and Sm suppressed Cytochrome c mRNA expression levels in cirrhotic rat liver regeneration at 24 h PHx ($p<0.05$) (Figure 6C). ESL and Sm suppressed Cytochrome c mRNA expression level in cirrhotic rat liver regeneration at 72 h PHx ($p<0.05$).

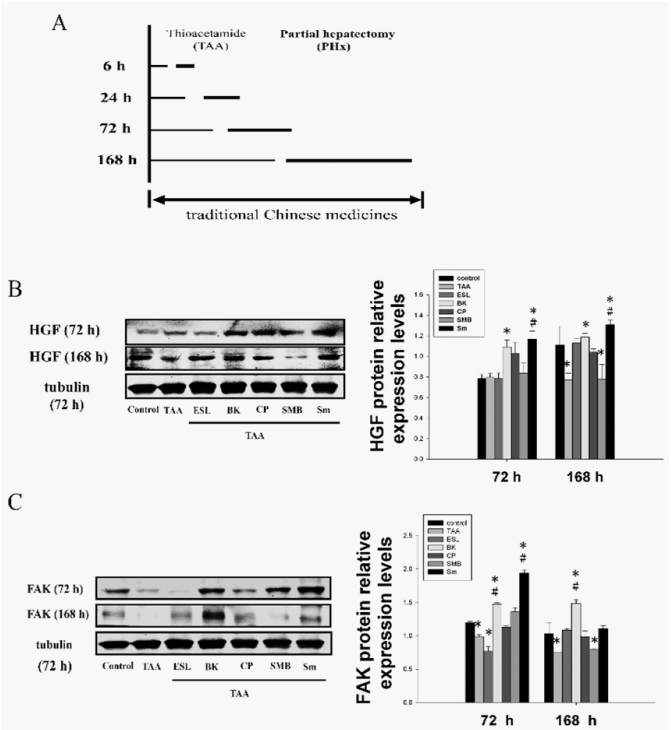

Figure 1: Protective effects of traditional Chinese medicines on the cirrhotic animal model with Thioacetamide (TAA). (A). Traditional Chinese medicines on the cirrhotic animal model with thioacetamide (TAA) at 6, 24, 72 and 168 h and then partial hepatectomy design. (B). HGF protein expression level was increased by BK and Sm at 72 and 168 h TAA liver-injured. (C). FAK protein expression level was increased by BK and Sm at 72 h TAA liver-injured, but only BK at 168 h TAA liver-injured. Equal amounts of lysate were separated by 12%SDS-PAGE by Western blot. Quantification of densitometry analysis of protein expression levels. All data are presented as means±SEM (n=6), *P<0.05 significantly compared with the corresponding control group. #P<0.05, significantly difference compared with the corresponding TAA- liver-injured. CP: Codonopsis Pilosula; BK: Bupleurum Kasi; ESL: Elephantopus Scaber L.; SMB: Salvia Miltorrhiza Bunge; Sm: Silymarin; TAA: Thioacetamide.

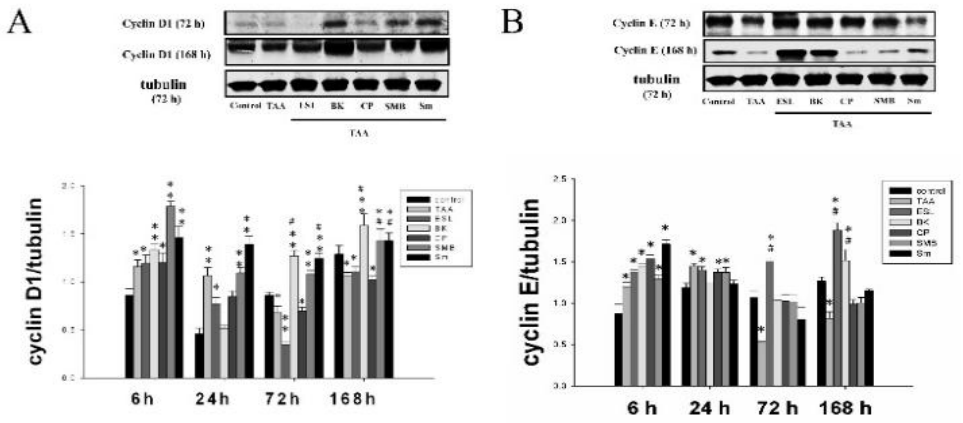

Figure 2: Traditional Chinese Medicine, Elephantopus Scaber L, Induced The Kinetics Of Cell Cycle Progression In The G1 Phase Transition In The Cirrhotic Rat Liver Model After TAA Treatment. (A).Traditional Chinese medicines, Bupleurum kasi, Salvia miltorrhiza Bunge, Silymarin, induced the cell cycle progression in the G1 phase in the cirrhotic rat liver model after TAA treatment. Cyclin D1 protein expression in cell cycle G1 phase at 6, 24, 72 and 168 h TAA-induced. Early 6 and 24 h TAA-injury, hepatocyte still maintained liver function, almost all Chinese herbal medicines improved Cycline D1 increased including TAA-injury treatment. After 72 to 168 h TAA-injury withdrawn, Chinese herbal medicines, BK, SMB and Sm, can improve Cyclin D1 increase. Densitometry analysis quantification of protein expression levels. (B). Cyclin E protein expression in the cell cycle S phase at 6, 24, 72 and 168 h TAA-induced. Early 6 and 24 h TAA-injury, hepatocyte still maintained liver function, almost all Chinese herbal medicines improved Cycline E increase including TAA-injury treatment. After 72 h TAA-injury withdrawal, Cycline E increased by ESL treatment, but at 168 h TAA-induced by ESL and BK have effects. Equal amounts of lysate were separated using 12%SDS-PAGE by Western blot. Densitometry analysis quantification of protein expression levels. All data are presented as means ± SEM (n=6), *P<0.05, **P<0.01 significantly compared with the corresponding control group. #P<0.05 significantly difference compared with the corresponding TAA- liver-injured. CP: Codonopsis Pilosula; SMB: Salvia Miltorrhiza Bunge; BK: Bupleurum Kasi; ESL: Elephantopus Scaber L; Sm: Silymarin. PHx: Partial Hepatectomy.

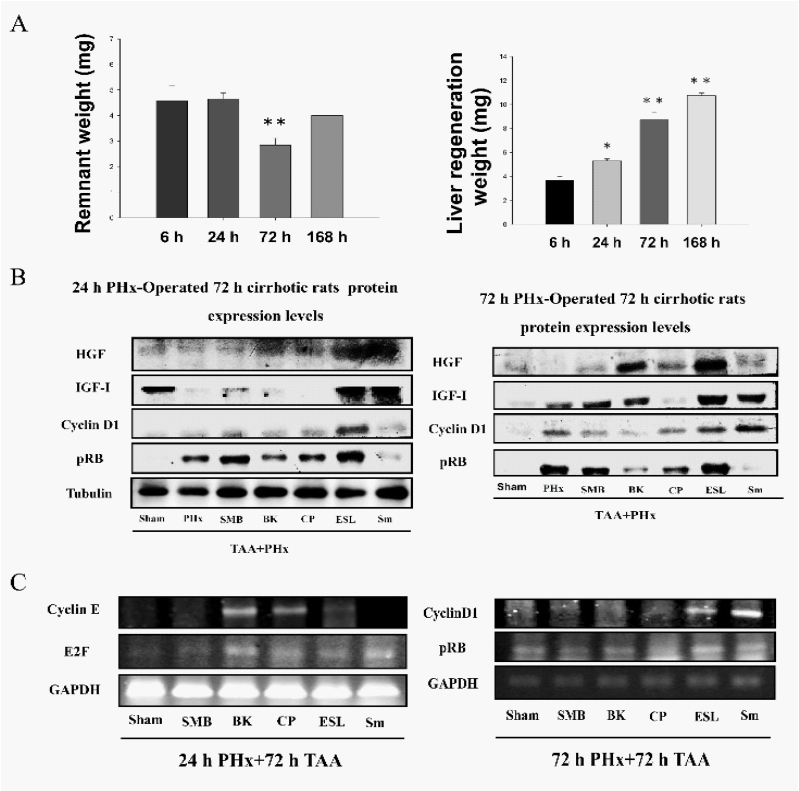

Figure 3: Effects Of Elephantopus Scaber L. (ESL) On Cirrhotic Rat Liver Regeneration Partial Hepatectomy. (A). Partial hepatectomy at 6, 24, 72 and 168 h PHx after TAA-induced cirrhosis. Remnant liver weight. All data are presented as mean ± SEM (n = 6), **p < 0.01 compared with 6 h PHx-TAA. Liver regeneration weight (mg) at 6, 24, 72 and 168 h cirrhotic rat liver regeneration after partial hepatectomy without treatment Chinese herbal medicines. All data are presented as means ± SEM (n=6) *P<0.05, **P<0.01, significantly difference compared with the corresponding 6 h PHx-TAA group. TAA; thioacetamide. (B). Western blot analysis of HGF, IGF-I, Cyclin D1 and pRb protein expression were increased by ESL at 24 and 72 h PHx 72 h TAA-induced cirrhotic rat liver regeneration. TAA; thioacetamide, PHx; partial hepatectomy. (C). Elephantopus scaber L. (ESL) maintaines hepatocytes in cell cycle G1 phase in cirrhotic rat liver regeneration after 24 h and 72 h PHx by RT-PCR. mRNA expression levels of Cyclin D1 expression in G1 phase and Cyclin E/E2F expression in S phase. ESL induced Cyclin D1 mRNA expression levels in G phase, but not found Cyclin E/E2F increases in cirrhotic rat liver regeneration at 24 h partial hepatectomy. In cirrhotic rat liver regeneration at 72 h partial hepatectomy, Cyclin D1/pRb mRNA expression level still increased by ESL and Sm, but not found E2F mRNA expression level increases. CP: Codonopsis Pilosula; SMB: Salvia Miltorrhiza Bunge; BK: Bupleurum Kasi; ESL: Elephantopus Scaber L; Sm: Silymarin. PHx: Partial Hepatectomy.

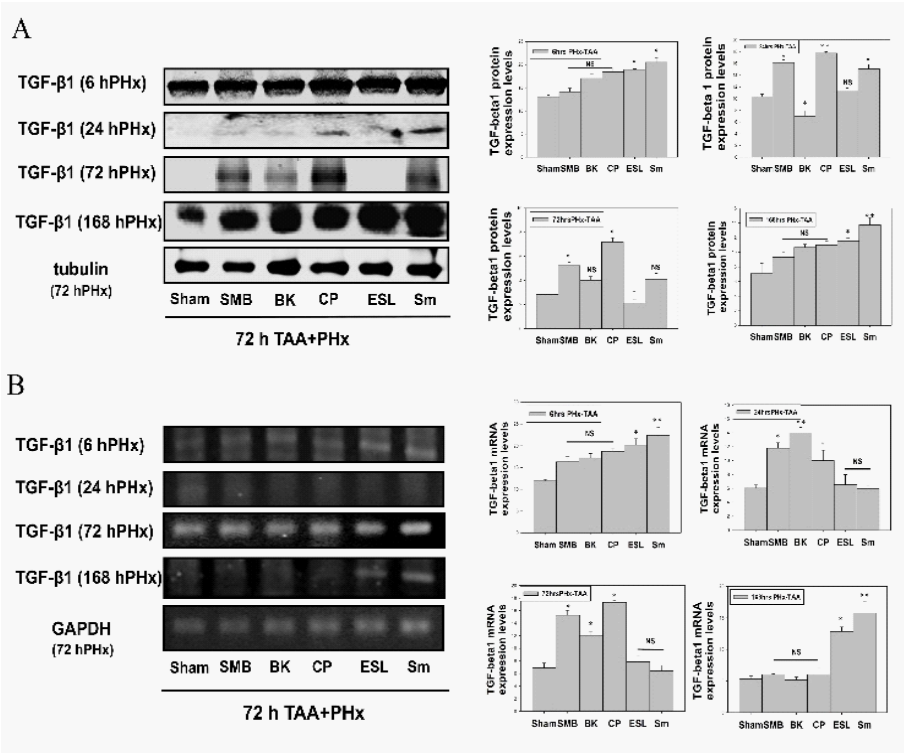

Figure 4: Effects Of Elephantopus Scaber L. (ESL) On Cirrhotic Rat Liver Regeneration After Phx Induced Transforming Growth Factor (TGF-B1) from G0 Priming Stage To G1 Phase By Western Blot And RT-PCR. (A) Western blot analysis of transforming growth factor (TGF-β1) protein expression levels in cirrhotic rat liver regeneration at 6, 24, 72 and 168 h partial hepatectomy treatment by Chinese herbal medicines. Quantification of densitometry analysis of transforming growth factor (TGFβ1) protein expression levels by western blotting analysis. ESL induced TGFβ1 increased at 6 h priming cell cycle G1 phase, then no effects on TGF-β1 at 24 h, then suppressed TGF-β1 at 72 h ($p<0.05$) to promote proliferation, and then promoted TGFβ1 increases at 168 h into the mitogenic response ($p<0.05$). Sm is positive control. All data are presented as mean ± SEM (n = 6), *p < 0.05, **P < 0.01 vs the corresponding Sham in cirrhotic rat liver regeneration after partial hepatectomy group. NS is no significant difference. CP, Codonopsis pilosula; SMB, Salvia miltorrhiza bunge; BK, Bupleurum kasi; ESL, Elephantopus scaber L; Sm Silymarin. PHx; partial hepatectomy. TAA; thioacetamide. (B). RT-PCR analysis of transforming growth factor (TGF-β1) mRNA gene expression levels in cirrhotic rat liver regeneration after 6, 24, 72 and 168 h PHx treatment by Chinese herbal medicines. Quantification of densitometry analysis of mRNA levels by RT-PCR. All data are presented as mean ± SEM (n = 6), *P < 0.05, **P < 0.01 vs the corresponding Sham in cirrhotic rat liver regeneration after partial hepatectomy group. NS is no significant difference. TGF-β1 mRNA expression was increased by ESL and Sm after TAA following 6 h PHx, then no effects after TAA following 24 and 72 h PHx to mitogenic effects for terminating the proliferative response, and then enhanced expression after TAA following 168 h PHx. CP, Codonopsis pilosula; SMB, Salvia miltorrhiza bunge; BK, Bupleurum kasi; ESL, Elephantopus scaber L; Sm Silymarin. PHx; partial hepatectomy. TAA; thioacetamide.

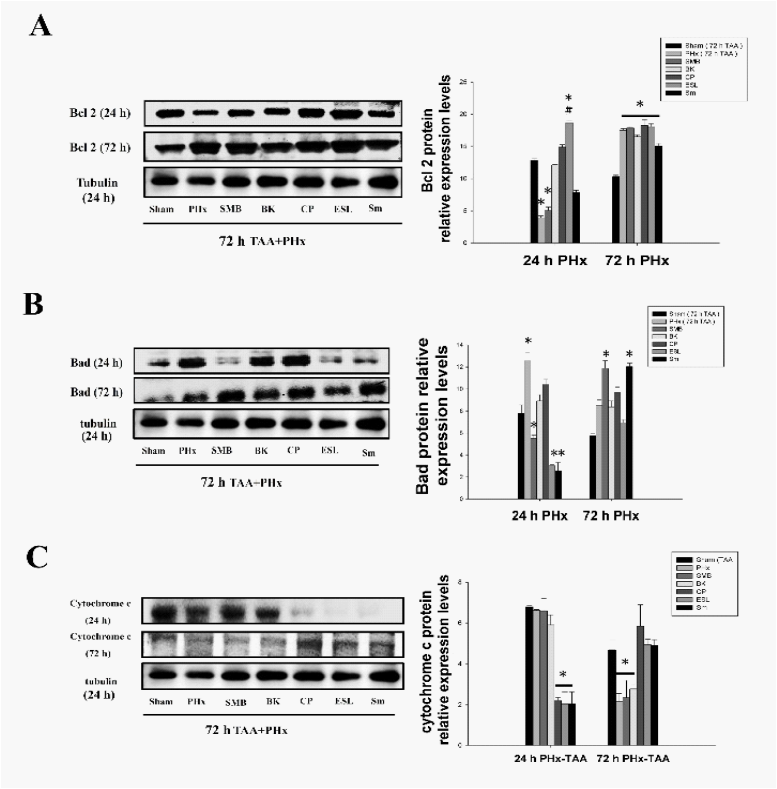

Figure 5: Effect Of Elephantopus Scaber L. (ESL) On Apoptosis Through Mitochondrial in Cirrhotic Rat Liver Regeneration Partial Hepatectomy Using Western Blotting Assay.
(A). Expression protein levels of Bcl 2 was increased by ESL treatment at 24 h PHx, but all Chinese medicines at 72 h PHx in 72 h TAA-induced cirrhotic rat liver regeneration. (B). Expression protein levels of Bad was decreased by ESL treatment at 24 h PHx in 72 h TAA-induced cirrhotic rat liver regeneration. (C). Expression protein levels of Cytochrome c was decreased by ESL treatment at 24 h PHx in 72 h TAA-induced cirrhotic rat liver regeneration. Equal amounts of lysate were separated using 12%SDS-PAGE using Western blot. Densitometry analysis quantification of protein expression levels. All data are presented as means ± SEM (n=6), *P<0.05 significantly compared with the corresponding control group. #P<0.05 significantly difference compared with the corresponding TAA- liver-injured. CP: Codonopsis Pilosula; BK: Bupleurum Kasi; ESL: Elephantopus Scaber L.; SMB: Salvia Miltorrhiza Bunge; Sm: Silymarin. TAA: Thioacetamide; PHx; Partial Hepatectomy.

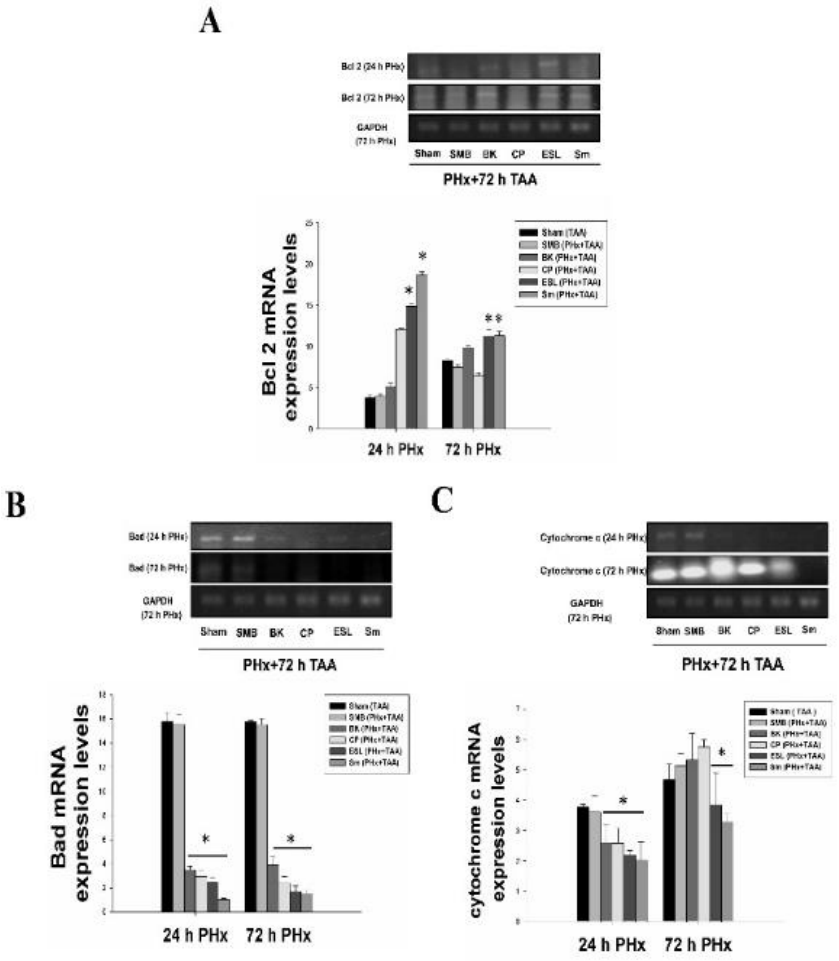

Figure 6: Effect of Elephantopus scaber L. (ESL) on apoptosis through mitochondrial in cirrhotic rat liver regeneration partial hepatectomy using RT-PCR assay.
(A). mRNA expression levels of Bcl 2 was increased by ESL and Sm at 24 h and 72 h PHx after TAA-induced cirrhotic rat liver regeneration. (B). mRNA expression level of Bad was decreased by BK, CP, ESL and Sm at 24 h and 72 h PHx after TAA-induced cirrhotic rat liver regeneration. (C). mRNA expression levels of Cytochrome c were decreased by BK, CP, ESL and Sm at 24 h PHx, by ESL and Sm at 72 h PHx after TAA-induced cirrhotic rat liver regeneration. Quantification of densitometry analysis of mRNA expression levels; All data are presented as means ± SEM (n = 6), *p<0.05 vs the corresponding Sham in cirrhotic rat liver regeneration after partial hepatectomy group. CP: Codonopsis Pilosula; SMB: Salvia Miltorrhiza Bunge; BK: Bupleurum Kasi; ESL: Elephantopus Scaber L; Sm: Silymarin;n; TAA; Thioacetamide; PHx; Partial Hepatectomy.

Table 1: Primer Pairssequences Used In Reverse Transcriptase- Polymerase Chain Reaction.

Primers		Sequence
Cyclin D1	forward primer	5'AGGAGACCATTCCCCTGACT3'
	reverse primer	5'TTCTTCCTCCACTTCCCCTT3'
Cyclin E	forward primer	5'ACCTACAGTGAAGATGCACACC3'
	reverse primer	5'CCTGTAGTTCTTGTTTCCTGCAC3'
pRb	forward primer	S'AGGAGGACTGTTCTCTAAGG3'
	reverse primer	5'GAGTGAGGTGTGTCTTCTGA3'
E2F	forward primer	5'AACATCCAGAACATCCAGTGGGTAGGCAG3'
	reverse primer	5'GGCTGTCAGTAGCCTCCAAG3'
Bcl 2	forward primer	5'CACCCCTGGCATCTTCTCCTT3'
	reverse primer	5'AGCGTCTTCAGAGACAGCCAG3'
Bad	forward primer	5'TAAGACTCACCTGGGTACTG3'
	reverse primer	5'GCATGTAGTCACTCTTCACC3'
cytochrome c	forward primer	5'ACAGCACGCTTGTGGAT3'
	reverse primer	5'GTCTTCAAGCAAGAGGACCA3'
TGF B1	forward primer	5'ACAGCACGCTTGTGGAT3'
	reverse primer	5'GTCTTCAAGCAAGAGGACCA3'
GAPDH	forward primer	5'GGGTGTGAACCACGAGAAAT3'
	reverse primer	5'CCACAGTCTTCTGAGTGGCA3'

Discussion

Liver fibrosis is a widespread alteration in chronic liver damage usually caused by alcohol and various toxins[19]. Although cirrhosis is the terminal stage of various liver diseases, the liver is one of the most complex organs possessing a potent orchestrated regeneration capacity. Set in the optimal mass in relationship to its body size, the liver induced its compensatory hyperplasia mechanisms. Traditional Chinese medicines have been used to treat liver disorders for thousands of years in the East. Now have become a promising therapy internationally for pathological liver conditions. In the present study, we presume that traditional Chinese medicines, Codonopsis Pilosula (CP), Salvia Miltorrhiza Bunge (SMB), Bupleurum Kasi (BK), Elephantopus Scaber L. (ESL) and Silymarin (Sm) may promote liver regeneration after PHx in cirrhotic rats. In this study, we are interested in the effects of Chinese herbal medicines on preserving enough liver remnants for normal body function after surgical resection to remove together with the surrounding cirrhotic liver tissue. Chronic liver diseases (i.e. liver fibrosis, cirrhosis, hepatitis and steatohepatitis) are the high risk factor of liver cancer [20]. Treatments for a hepatic tumor suggested surgery to remove the growth and with the goal of preventing or arresting metastatic cancer. We expected to compile and discusses the molecular biological analytical method of herbal medicines for liver protection. We established a Thioacetamide (TAA)-induced liver cirrhotic model elucidated the protective effects of traditional Chinese herbal medicines on the mechanisms underlying liver regeneration. We make an establishment of the cirrhotic animal model with thioacetamide (Table 2). Liver damage at different time, but recover at withdrawal time. Results showed BK and Sm induced HGF and FAK, but Cyclin D1 increased by BK, SMB and Sm after 72 and 168 h TAA-induced, but ESL induced Cyclin E protein expression (Figure 1 & Figure 2). We think maybe its function is stronger in the G1 phase. However, morphological restoration and function restoration of the cirrhotic liver was accelerated by partial hepatectomy. Most commonly, when the liver is injured it attempts to repair the injured site, referred to as internal scar tissue as quickly as possible [21,22]. Partial hepatectomy during liver regeneration induced for maintaining homeostasis. After surgery hepatocyte need to growth and to maintain liver mass. However, native hepatocyte proliferation function can not maintain the integrated whole liver function after PHx [23-25]. Liver regeneration weight after PHx administration increased from 6 to 168 h (Figure 3). We found that ESL stimulated HGF, IGF-I, Cyclin D1 and pRB into cell cycle G1 phase induced DNA synthesis in 72 h cirrhotic rat liver regeneration after 24 and 72 h PHx (Figure 3). ESL has shown maintain effects in treating nearly every known form of liver disease during cirrhosis and liver damage due to drugs [26]. That is how they are used today. ESL has shown the TGF-β1 mechanisms of priming cell cycle G0 phase and stopped hepatocytes proliferation in cirrhotic rat liver regeneration after PHx (Figure 4). The initiation of DNA synthesis occurs in hepatocytes after surgery [27]. ESL make cell cycle accelerated in G1 phase to improve PHx-induced cell cycle delayed. However, ESL not only enhanced the G1 cell cycle phase, but also induced anti-apoptosis cells and anti-apoptosis protein, Bcl2, and suppressed apoptosis, Bad and cytochrome c, in 24 and 72 h after PHx administration in cirrhotic rats (Figure 5 and Figure 6). PHx is a cell cycle dependent regulator with a potential physiological role in G1 progression [28-30]. In summary, our data suggested that ESL may induce Bcl 2 protein expression level increases and suppression Bad and Cytochrome c protein expression. We found that cell death occurred only at after TAA-induction in cirrhotic rats. We think PHx-induced liver regeneration terminated after thioacetamide treatment. traditional Chinese Medicines, ESL could complementary protection in cirrhotic rat liver regeneration PHx. In conclusions, Elephantopus Scaber L. (ESL) have protection function in the regenerating cirrhotic liver after PHx. ESL have been used to treat liver disorders for thousands of years and now become a promising therapy internationally for pathological liver conditions. Especially, the regeneration induced by surgical injury is an orchestrated response. Overall, we suggest ESL has shown the mechanisms of many anti-cirrhotic drugs acting as hepatoprotective agents in vivo.

Acknowledgments

This work was supported by a grant from the National Science Council, Republic of China.

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