Hepatoprotective Effects of Chinese Medicine Herbs Decoction on Liver Cirrhosis in Rats

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Hepatoprotective and curative activities of aqueous extract of decoction containing 10 Chinese medicinal herbs (HPE-XA-08) were evaluated in Sprague–Dawley albino rats with liver damage induced by thioacetamide (TAA). These activities were assessed by investigating the liver enzymes level and also histopathology investigation. Increases in alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) levels were observed in rats with cirrhotic liver. No significant alterations of the liver enzymes were observed following treatment with HPE-XA-08. Histopathology examination of rats treated with HPE-XA-08 at 250mg/kg body weight, however, exhibited moderate liver protective effects. Reduced extracellular matrix (ECM) proteins within the hepatocytes were noted in comparison to the cirrhotic liver. The curative effects of HPE-XA-08 were observed with marked decrease in the level of ALP (more than 3x) and level of GGT (more than 2x) in cirrhotic rat treated with 600 mg/kg body weight HPE-XA-08 in comparison to cirrhotic rat treated with just water diluent. Reversion of cirrhotic liver to normal liver condition in rats treated with HPE-XA-08 was observed. Results from the present study suggest that HPE-XA-08 treatment assisted in the protection from liver cirrhosis and improved the recovery of cirrhotic liver.

1. Introduction

Chronic liver disease is the ninth leading cause of mortality in Western and developing countries [1]. The disease resulted from chronic proinflammatory injuries, which could cause progressive fibrosis causing the liver to scar and eventually becomes cirrhotic. There are many factors that could lead to chronic liver injury and among them are viral infections such as hepatitis C infection, chronic alcoholism, and autoimmune diseases and also due to drug or substance abuses. Five percent of persons with liver cirrhosis could progress to develop liver cancer [2].

To date, there is no specific treatment for liver cirrhosis. Sufferers are treated to reduce the complications due to the damaged liver from exacerbating. The treatments are often expensive especially for those in developing countries where there is high rate of liver cirrhosis in the population. Due to these factors, treatment using ethnobotanical approach has gained popularity as an alternative cost-effective approach [1, 3]. Among the ethnobotanical approaches, the Chinese herbal medicine has been widely applied. It serves as alternative complementary medicine, probably due to the presence of complete pharmacopeia of the herbs established over more than 5000 years of traditional use. Currently, in many healthcare facilities in China, traditional Chinese medicine is being applied in complement to Western medicine [4]. Typical in most traditional Chinese medicines, the formulation consists of multiple herbs concoction. It has been suggested that the combination of herbs with the various combinations of natural ingredients may produce synergistic
of the equivalent volume. Blood was collected from each rat before and after the treatment. The treatment was performed for 30 days.

2.4. Hepatic Biochemical Evaluation. Blood of each rat was collected via tail vein before the treatment and at the end of the treatment regime. The blood was collected in tube containing EDTA and analyzed for the liver function enzymes serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) in the Clinical Diagnostic Laboratory (CDL) at University Malaya Medical Centre (UMMC). Immediately after the final blood collection, the rats were euthanized and the organs were harvested for histological investigation.

2.5. Histopathological Analysis. Liver samples harvested from the rats were washed with the normal saline and immediately fixed in 10% buffered neutral formalin for 48 hours. Samples of 5-micron thickness were prepared, processed in alcohol-xylene series, and stained with alum-paraflax and eosin, prior to histopathological examination.

2.6. Statistical Analysis. Results were presented as mean ± SEM of six animals in each group. The data were subjected to one-way ANOVA followed by Bonferroni’s posttest. \( p < 0.05 \) was considered statistically significant. Analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, USA).

3. Results

3.1. Prevention of TAA-Induced Liver Cirrhosis. The effects of HPE-XA-08 on serum ALT, AST, ALP, and GGT and bilirubin activities in rats from all treatment groups were shown in Table 1. It is observed that the serum ALT and AST activities in all rat groups, Groups 1P, 2P, 3P, and 4P, did not show any significant differences following the preventive treatment regime. It was noted that the serum ALT activity in rat group treated with HPE-XA-08 showed lower ALT activity with value of 76.0 ± 4.416 IU/L (Group 2P) and 69.40 ± 7.756 IU/L (Group 4P) in comparison to those injected with normal saline only (Groups 1P and 2P). The highest level of ALP was observed in Groups 3P and 4P in comparison to those injected with normal saline only (Groups 1P and 2P). The highest level of ALP was observed in Group 3P with value of 223.3 ± 11.16 IU/L, followed by Groups 4P, 2P, and 1P with values of 222.4 ± 10.41 IU/L, and 215.0 ± 12.84 IU/L, respectively. Serum ALP level in rats injected with TAA (Groups 3P and 4P) were higher in comparison to those injected with normal saline only (Groups 1P and 2P). The highest level of ALP was observed in Group 3P with value of 215.0 ± 10.41 IU/L. The ALP level in rats injected with TAA and treated with HPE-XA-08 (Group 4P) was observed to be lower than those treated with water (Group 3P) with value of 277 ± 4.722 IU/L. The serum GGT levels in rats injected with TAA (Groups 3P and 4P) were significantly higher in comparison to the control groups (Groups 1P and 2P). Serum GGT levels in TAA control group (Group 3P) were the highest with value...
Table 1: Effect of HPE-XA-08 on blood biochemicals related to liver damage (ALT, AST, ALP, and GGT) for observation of protective activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
<th>GGT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline + H2O (1P)</td>
<td>80.00 ± 4.175</td>
<td>237.3 ± 12.84</td>
<td>173.6 ± 7.807</td>
<td>2.286 ± 0.4206</td>
</tr>
<tr>
<td>Normal saline + HPE-XA-08 (2P)</td>
<td>76.00 ± 4.416</td>
<td>223.3 ± 11.16</td>
<td>170.3 ± 23.68</td>
<td>3.750 ± 0.4787</td>
</tr>
<tr>
<td>TAA + H2O (3P)</td>
<td>88.50 ± 12.75</td>
<td>215.0 ± 13.64</td>
<td>375.0 ± 10.41*</td>
<td>37.50 ± 1.857*</td>
</tr>
<tr>
<td>TAA + HPE-XA-08 (4P)*</td>
<td>69.40 ± 7.756</td>
<td>222.4 ± 5.715</td>
<td>277.0 ± 4.722</td>
<td>30.80 ± 3.980</td>
</tr>
</tbody>
</table>

* Levels of enzymes between 1P and 3P were compared to show the effects of TAA on the level of liver enzymes; * indicates p < 0.05.

# Levels of enzymes between 1P and 2P were compared to show the effects of HPE-XA-08 on the level of liver enzymes but did not show statistically significant value (p > 0.05).

+ Levels of enzymes between 3P and 4P were compared to show the effects of HPE-XA-08 on preventing TAA-induced liver cirrhosis but did not show statistically significant value (p > 0.05).

3.2. Treatment of TAA-Induced Liver Cirrhosis. The possibility for HPE-XA-08 to reverse liver cirrhosis in rats was also evaluated. All rats were inoculated with TAA to induce liver cirrhosis, except for rat group 1T which was inoculated with normal saline to serve as control group (normal rat). Following 12 weeks of induction of liver cirrhosis with TAA,
the rats were either treated with distilled water (Group 2T) or with 600 mg/kg HPE-XA-08 for 30 days (Group 3T). The hepatic biochemical activities in normal and cirrhotic rats were evaluated before and after the treatment regime. The hepatic enzyme levels of ALT, AST, ALP, and GGT were measured (Table 2). It was noted that the AST, ALP, and GGT levels decreased after the 30-day treatment in all rat groups. The ALT level, however, was noted to increase in the cirrhotic rat groups (Groups 2T and 3T) following the 30-day treatment with increment from 62 ± 0.636 IU/L to 75 ± 0.577 IU/L in Group 2T and 63 ± 1.201 IU/L to 69 ± 0.441 IU/L in Group 3T. Withdrawal of TAA helped to significantly reduce the level of ALP and GGT in Group 2T although the rats were treated with just water. In rats treated with HPE-XA-08 (Group 3T), further decrease of ALP and GGT levels with reduction from 368 ± 0.882 IU/L to 102 ± 2.333 IU/L and 39 ± 0.296 IU/L to 15 ± 0.441 IU/L for ALP and GGT, respectively, was observed. The ALT level was reduced significantly following treatment with HPE-XA-08 with reduction from 179 ± 0.882 IU/L to 158 ± 0.882 IU/L. Unlike those treated with HPE-XA-08, the AST level in the cirrhotic rat treated with water was 180 ± 1.155 IU/L and 175 ± 0.667 IU/L before and after treatment, respectively. Macroscopic observation of the liver from the normal control rat (Group 1T) showed the liver to be dark red in color with smooth homogenous surface (Figure 2(a)). In the cirrhotic control group treated with water (Group 2T), although the TAA has been discontinued, the liver was slightly brownish with macronodular structure and irregular nonhomogenous surface (Figure 2(b)). For the cirrhotic rats treated with HPE-XA-08, the color of the liver was more reddish compared to the group treated with only water (Figure 2(c)). The liver surface showed no obvious nodular structure and the surface of the liver was comparable to the normal liver.

4. Discussion

Hepatic cirrhosis, a consequence of hepatic fibrosis, is characterized by exaggerated production of extracellular matrix properties (ECM) [12]. Hepatic cirrhosis induced by thioacetamide (TAA) lead to steatosis, which is associated with the aggravation of lipid peroxidation and depletion of antioxidant status [13].

In the present study, liver damage is manifested by increases in serum ALP, GGT, and bilirubin levels. TAA-induced liver cirrhosis, however, was usually not characterized by high serum ALT and AST, unlike those intoxicated by CCl₄ and paracetamol [14, 15]. Further, it has been reported that the severity of liver injury does not correlate with the level of liver enzyme elevation [16]. From our observation on the preventive effects of HPE-XA-08 on liver cirrhosis, although the differences between the level of liver enzymes in those untreated and those treated with HPE-XA-08 were not significant, except for level of GGT, the macroscopic and histologic examinations supported that treatment with HPE-XA-08 protected the liver against severe damage. Based on our findings, ALP and GGT could be employed as indicator in evaluating liver damage induced by TAA; however, the enzyme levels may not corroborate with the physical condition of the liver. The histological observation of the liver of rats given TAA was consistent with that reported by Bruck et al. [12], where increase in ECM was noted. In this study, however, we did not determine the mechanisms of action of the herbal concoction (HPE-XA-08) in providing the protective effects on the liver against induced damage. We postulated that the HPE-XA-08 decoction may affect the collagen content in the liver by either increasing the collagen synthesis, or reducing the collagen degradation, which was evidenced by the resolution of the fibrotic lesion and ECM in
Table 2: Blood enzymes related to liver damage (ALT, AST, ALP, and GGT) for observation of curative activity conferred by HPE-XA-08.

<table>
<thead>
<tr>
<th>Liver enzyme</th>
<th>Normal rat (Group 1T)</th>
<th>Cirrhotic rat treated with distilled water (Group 2T)</th>
<th>Cirrhotic rat treated with 600 mg/kg HPE-XA-08 (Group 3T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of liver enzyme (before treatment; IU/L)</td>
<td>Concentration of liver enzyme (after treatment; IU/L)</td>
<td>Concentration of liver enzyme (before treatment; IU/L)</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>77 ± 1.528</td>
<td>64 ± 0.318&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62 ± 0.636&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>203 ± 3.512</td>
<td>184 ± 1.856&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180 ± 1.155&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>198 ± 1.856</td>
<td>96 ± 1.155&lt;sup&gt;a&lt;/sup&gt;</td>
<td>368 ± 1.155&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G-Glutamyl transferase (GGT)</td>
<td>2 ± 0.333</td>
<td>1 ± 0.067</td>
<td>38 ± 0.333&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> p < 0.05 when comparing level before and after treatment.

<sup>b</sup> p < 0.05 when compared with normal control (Group 1T).

<sup>c</sup> p < 0.05 when compared with untreated cirrhotic group (Group 2T).
the liver of HPE-XA-08-treated rat observed macroscopically and histologically. The liver harvested from cirrhotic rat treated with HPE-XA-08 showed accelerated resolution of existing fibrosis. The morphology of the liver has reverted to almost comparable to the normal liver (Figure 2(c)). This finding was consistent with reports by Chen et al. [17] and Park et al. [18] that demonstrated that 2 out of 10 components within HPE-XA-08 and Gardenia jasminoides and Scutellaria baicalensis play a role in attenuation of collagen accumulation and apoptosis of hepatic stellate cells (HSCs), respectively, which help in resolution of liver fibrosis. Other than that, the resolution of liver damage may also be assisted by extract of Ligustrum lucidum which was reported to induce apoptosis and cell senescence in human hepatocellular carcinoma [19].

Liver fibrosis and cirrhosis were earlier thought of as irreversible processes. However, recent clinical and experimental evidences suggest that the process can be reversed [2, 20]. To assess the potential curative effects of HPE-XA-08 on liver cirrhosis, the hepatotoxic TAA was removed during the treatment process. We noted that although TAA has been withdrawn for 30 days, the liver nodulation could still be observed (Figure 2(b)); however, the liver condition was not as nodulated as observed in the liver with the presence of TAA (Figure 1(e)) suggesting possible arrest of progressive liver damage. Previous report showed that TAA-induced steatosis was due to accumulation of lipids within the hepatocytes [21]. Our observation in the rats’ cirrhotic liver was consistent with this report (Figure 2(b)), where lipid nodules could be observed in the cirrhotic liver of rats treated with water (H2O). Amelioration of hyperlipidemic liver observed in our study could be contributed by constituents present in Chrysanthemum morifolium and Artemisia scoparia (within HPE-XA-08 decoction), which has been described to attenuate high-fat milk-induced fatty liver and reduce the liver lipid accumulation, respectively [22, 23]. In the assessment of liver enzyme, the level obtained before the treatment was used as a baseline to be compared to the enzyme level after the treatment. It was noted that the levels of liver enzyme in normal rat decreased after 30 days of treatment with water. This could be related to the age of the rat, since age does affect the liver enzymes [24], which indicated that observation of liver enzyme level alone was insufficient in evaluating hepatoprotective effects conferred by any herbal supplement and needed to be complemented with biopsy or histology.

Most of the previous studies on the antifibrotic effects were performed on single plant/herb extract. In this study, the hepatocellular injury and fibrosis in rats were treated orally with decoction consisting of 10 medicinal Chinese herbs, named HPE-XA-08. Other than the 5 plants/herbs within HPE-XA-08 that have been mentioned above, the remaining 4 plants/herbs in the decoction, Phellodendron amurense, Poria cocoa, Bupleurum B. scorzoneraefolium, and Taraxacum mongolicum, scientifically were reported to exert anticarcinogenic and antitumor properties [25–28]. Based on our findings, we conclude that the decoction consisting of the 10 medicinal Chinese herbs, named HPE-XA-08, possesses potential hepatoprotective and curative effects against damaged liver. Further investigations, however, are needed to identify the hepatoprotective mechanisms conferred by this aqueous herbal preparation. The present finding provides scientific evidence that the mixture of these 10 Chinese traditional herbs preparation possessed ethnomedical properties in maintaining liver function and could be developed further as ethnomedicine for liver injuries.

List of Abbreviations
ALT: Alanine aminotransferase
ALP: Alkaline phosphatase
AST: Aspartate aminotransferase
ECM: Extracellular matrix
GGT: Gamma-glutamyl transferase
H2O: Water
IP: Intraperitoneal
SEM: Standard error of mean
TAA: Thioacetamide.

Competing Interests
Tong-Hye Lim is the herbalist in Herbitec (M) Sdn Bhd. Sazaly AbuBakar is the Scientific Consultant for Herbitec (M) Sdn Bhd. Nor Aziyah Mat-Rahim and Nur-Asyura Nor-Amdan have no conflict of interests.

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References


