Hepatoprotective Effect Of Crude Aqueous Leaf Extract Of Fig Tree, Ficus Benjamina, On Ethanol-Induced Liver Damage In Mice

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Abstract: Alcoholic abuse remains to be the most common cause of liver cirrhosis with significant morbidity and mortality worldwide. Herbal supplements are being used to prevent damage in excessive alcohol intake and including hepatitis from other causes as hepatoprotective agents. Fig tree is currently being utilized in studies as a potential candidate for hepatoprotection but with limited success. This study determined the hepatoprotective effect of crude aqueous leaf extract of fig tree, Ficus benjamina, on ethanol-induced hepatotoxicity in mice. In this study, fifteen Balb/c mice were assigned to negative control, positive control and treatment groups which received distilled water, Silymarin, and F. benjamina crude aqueous leaf extract respectively on day 0 to day 14. Ethanol-induced hepatotoxicity was done on day 7 to day 14 using ethanol given by oral gavage. Assessment of liver function and histology was done with the use of alanine aminotransferase (ALT) assay and histopathological study respectively. Results showed significant reduction of ALT levels in the treatment (52.40 U/L) and the positive control groups (42.58 U/L) as compared with the negative control group with a mean of 196.88 U/L ($P<0.05$). The difference between the positive and treatment groups was not significant ($P>0.05$). The degree of hepatic injury was significantly severe in the negative control group than with the treatment and positive control groups ($P<0.05$). On the other hand, the degree of hepatic injury showed no significant difference between the positive and treatment groups ($P>0.05$). Thus, the crude aqueous leaf extract of F. benjamina has hepatoprotective property on ethanol-induced hepatotoxicity in mice, similar to Silymarin. F. benjamina, as an ornamental plant, may be a source of phytochemical with potential pharmaceutical and functional activities.

Index Terms: Ethanol-induced hepatotoxicity, Ficus benjamina, Mice

1 INTRODUCTION

Alcoholic liver disease is a range of conditions, which involves damage to the liver and its function induced by alcohol abuse. According to the latest World Health Organization (WHO) data, liver disease deaths in the Philippines reached 7,232 or 1.72% of total deaths. Cirrhosis is the final and irreversible form of alcoholic liver disease. Short-term ingestion of as much as 80 grams of alcohol (six beers or 8 ounces of 80-proof liquor) over one to several days generally produces mild, reversible hepatic fatty degeneration. Exposure to alcohol causes fatty liver, dysfunction of mitochondrial and cellular membranes, hypoxia, and oxidative stress [1]. Herbal supplements containing milk thistle, licorice root, bupleurum and cordyceps might offer protective or reparative actions to the liver. These herbs, along with white peony root, are sometimes recommended to help in treating cirrhosis of the liver.

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Andrographis, artichoke leaf, turmeric, schisandra, sweet potato, phyllanthusm, picrorhiza, noni, dandelion and beet leaf are also thought to help support the liver or assist in treating liver diseases. Silymarin, a unique flavonoid complex, contains silybin, silydianin, and silychrisin [2]. It is used in treating liver diseases. However, no conclusive scientific evidence supports the use of any of these herbal supplements for treating or preventing liver problems. Hence, they are simply used as natural products as food supplements with no specific therapeutic claims [3]. Silymarin, derived from the milk thistle plant. As an antioxidant, it scavenges free radicals that can damage cells exposed to toxins. It also increases glutathione in the liver by more than 35% in healthy subjects and by more than 50% in rats. High level of glutathione in the liver increases its capacity for detoxification. It also stimulates protein synthesis in the liver, which results in an increase in the production of new liver cells to replace the damaged ones [4]. Presently, this is widely used commercially as a food supplement in liver protection from alcohol intake under the proprietary brand Liveraide®. Ficus sp. of the family Moraceae, more commonly known as the fig tree, is a potential candidate for hepatoprotective property. It consists of over 800 species that are of great importance in the pharmaceutical studies. One notable species is F. carica (common fig), which is claimed to be useful in liver and spleen disorders, to cure piles and in the treatment of gout [5]. Chemical examinations reveal the presence of substances that may act as anticancer and antiproliferative agents [6]. Another species known as F. hispida, was reported to possess significant hepatoprotective property against carbon tetrachloride (CCl_4)-induced liver damage [7]. In a similar study conducted by Kanaujia et al. (2011), ethanolic extract of F. benjamina was also determined to possess hepatoprotective activity against CCl_4-induced hepatotoxicity [8]. Ficus benjamina, a known ornamental plant, has been claimed to be useful in the treatment of certain skin disorders, stomachache and dysentery (see Figure 1). Its fruit

was reported to have significant anti-tumor and antibacterial activity [9]. Therefore, it possesses promising pharmaceutical properties for the treatment of different diseases. The purpose of this research was to assess the hepatoprotective effect of crude aqueous leaf extract of F. benjamina on ethanol-induced hepatotoxicity in mice.

Figure 1. Representative of the plant Fig tree, Ficus benjamina

2 Materials and Methods

This study was approved by the Pamantasan ng Lungsod ng Maynila, College of Medicine Research Committee and conducted at the Biochemistry Laboratory of the College of Medicine, Pamantasan ng Lungsod ng Maynila (PLM-CM) and registered with the Research Grants Administration Office of the National Institutes of Health, University of the Philippines-Manila.

2.1 Plant Preparation and Extraction

The leaf sample of Ficus benjamina was obtained from Villamor Air Base, Pasay City and was authenticated by the National Museum, Manila. The leaves were rinsed thoroughly before use and dried at room temperature for seven days under the shade. The dried leaves were pulverized using a mortar and pestle. The powdered leaf sample was stored in an airtight container until further use. The shade-dried powder was mixed directly with 100 ml distilled water to produce a 500 mg/ml aqueous extract. After two hours of maceration, the solution was centrifuged for 15 minutes at 10,000 rpm. The supernatant was obtained and placed on a clean Falcon™ tube. The extract was stored at 4°C to 8°C until further use.

2.2 Animal Care and Preparation

Fifteen male Balb/c mice were used in this study weighing approximately 15 to 20 grams each and about 8 to 10 weeks old. These animals were obtained from the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines-Los Baños. The use and care of the mice followed the guidelines approved by the Philippine Association of Laboratory Animal Science (PALAS). The mice were given access to standard animal pellets and distilled water ad libitum.

2.3 Hepatotoxicity Induction with Ethanol

The mice were divided into three groups. Negative control group (n=5) received distilled water 0.3 ml via oral gavage. Positive control group (n=5) received Silymarin (Liveraide®) 100 mg per kilogram body weight given via oral gavage. Treatment group (n=5) received crude aqueous leaf extract of Ficus benjamina with a stock dose of 500 mg/ml given via oral gavage at 500 mg per kilogram body weight. All mice were given the above treatments on day 0 up to day 14. Induction of hepatotoxicity was done using 0.3 ml 50% (v/v) ethanol, which was given daily via oral gavage on day 7 up to day 14. Oral gavage of ethanol was done two hours after the administration of the treatment as specified above to each group. The experiment was terminated on day 14.

2.4 Assessment of Liver Function

On day 14, blood samples were collected through cardiac puncture using tuberculin syringe with Gauge 21 needle under anesthesia using Pentobarbital 0.4 mg/kg body weight given intraperitoneally and were allowed to clot at room temperature. The serum was obtained upon centrifugation of blood samples at 15,000 rpm for 20 minutes. Only 750 microliters of whole blood was taken. Samples were sent for alanine aminotransferase (ALT) determination to Hi-Precision Laboratory, Ermita, Manila using an automated biochemical analyzer employing Kinetic Method using the NAD/NADPH oxidative and reductive enzyme reaction coupled with the transaminase reaction.

2.5 Histopathologic Studies

After blood collection, the mice were sacrificed by cervical dislocation and the abdomen was cut open to remove the liver en toto. The liver samples were sliced in one-centimeter thickness and were fixed in 10% neutral buffered formaldehyde and sent for histopathological examination using Hematoxylin and Eosin (H&E) staining to the Pamantasan ng Lungsod ng Maynila-College of Medicine Pathology Laboratory. The two pathologists were blinded to the group assignment and assessed the degree of hepatic injury. The average of the grade given by the two blinded-pathologists was used as the grade assignment for each sample. The degree of hepatic injury was assessed for normal to significant distortion of the liver architecture, presence of foci of necrosis and presence of mild to moderate steatosis using the grading system as shown in Table 1.

TABLE 1

*HISTOPATHOLOGIC GRADING BY HISTOPATHOLOGIC STUDIES**

	Grading Microscopic Findings
0	Normal hepatic architecture
1	Mild distortion of liver parenchymal architecture with minimal focal necrosis
2	Mild distortion of liver parenchymal architecture with several foci of liver necrosis
3	Moderate distortion of liver parenchymal architecture with multiple foci of liver necrosis
4	Severe distortion of liver parenchymal architecture with multiple foci of liver necrosis

**The average of the grades obtained was considered as the hepatotoxicity grade.*

2.6 Statistical Analysis

The age and weight of all mice were determined and statistical analysis was done to ensure homogeneity. The ALT results and hepatotoxicity grades were presented in mean for each group. Statistical analysis for parametric variable was done using one-way ANOVA while for non-parametric variable was done using Wilcoxon Sum-Rank test. The level of significance was set at $P<0.05$, with a confidence level of 95%. Portable IBM SPSS Statistics version 19 software was used for the statistical analysis.

3 Results

The results of the alanine aminotransferase (ALT) assay showed significant hepatic damage to the negative control group (mean = 196.88 U/L) as compared to the positive control group (mean = 42.58 U/L) and the treatment group (mean = 52.40 U/L) ($P <0.05$) as shown in Figure 2. The mean ALT assay of the positive control group was 42.58 U/L, which was lower than the mean ALT assay of the treatment group, which was 52.40 U/L. However, the difference was not statistically significant ($P>0.05$). Histopathological studies done on the liver further supported the use of ethanol as a good model for hepatotoxicity studies as used in the present study. The representative photomicrographs of the hepatotoxicity of each group are presented in Figure 3. The hepatotoxicity grading of the three groups was determined based on the microscopic observations obtained. The negative control group showed significantly higher grade of 3 as compared with the positive control group with a grade of 1 and the treatment group with a grade of 2 ($P<0.05$). The hepatotoxic effect of ethanol was significantly reduced in the positive control group, which presented with a hepatotoxicity grade of 1, having mild distortion of liver parenchymal architecture with minimal focal necrosis. The hepatocytes in the positive control group showed a normal histology of the liver with no significant difference when compared with the treatment group (as shown in Figure 2B). A mild distortion of liver parenchymal architecture was observed in F. benjamina extract-treated mice while a moderate distortion of liver parenchymal architecture was observed in untreated mice. This showed that the hepatic histopathology of F. benjamina extract-treated mice was comparable with the standard Silymarin.

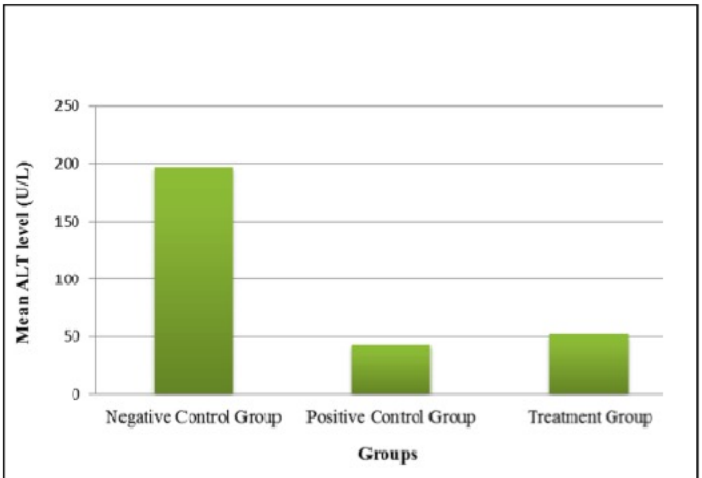

Figure 2. Means of alanine aminotransferase (ALT) levels measured in negative control group (distilled water), positive control group (Silymarin) and treatment group (*F.benjamina* extract).

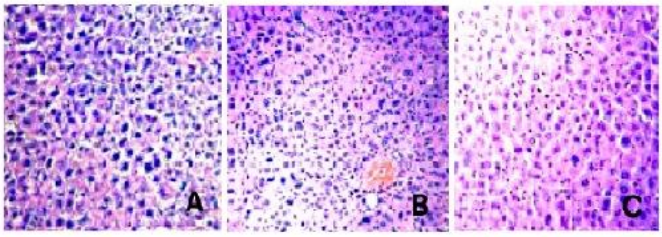

Figure 3. Photomicrographs of ethanol-induced hepatotoxicity in mouse liver treated with (A) distilled water, (B) Silymarin and (C) *F. benjamina* extract showing the degree of hepatic injury induced by ethanol (4,000x).

4 Discussion

This study validated the use of ethanol to induce hepatic injury as a model in hepatotoxicity study in animal studies. Alcohol is primarily metabolized in the liver and to a lesser extent in the gastrointestinal tract. In the liver, alcohol is metabolized by the action of alcohol dehydrogenase and by xenobiotic metabolism through cytochrome P-450 mainly through its isoform CYP2E1. Alcohol is converted to acetaldehyde via the action of alcohol dehydrogenase while acetaldehyde is converted to acetate via the action of acetaldehyde dehydrogenase participating in various metabolic pathways as two-carbon molecule. CYP2E1 also converts alcohol to acetaldehyde [10]. Alcohol exerts its hepatotoxicity through various interconnected pathways. Alcohol dehydrogenase, acetaldehyde dehydrogenase and CYP2E1 increase the production of acetate from alcohol, which reduce the nicotinamide adenine dinucleotide (NAD) to reduced nicotinamide adenine dinucleotide (NADH). The decreased NAD/NADH ratio inhibits gluconeogenesis and fatty oxidation, which channels the acetate to fatty acid synthesis causing liver fatty degeneration. Although an irreversible reaction, fatty liver may be the focal point for alcoholic liver disease when alcoholic intake is not abated. Alcohol also produces liver damage through the action of reactive oxygen species (ROS) producing hepatocyte necrosis, apoptosis, inflammation and fibrosis. CYP2E1 produces free radicals through the oxidative conversion of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP. Prolonged alcohol exposure activates the hepatic macrophages, which produce and release tumor necrosis factor-alpha (TNF-α). TNF-α stimulates the mitochondria to produce more ROS causing hepatic necrosis and apoptosis. This process continues if alcohol intake is unabated as a vicious cycle leading to end-stage liver disease. Similarly, acetaldehyde when combined with cellular proteins becomes antigenic creating a vicious cycle of inflammation leading to liver fibrosis and cirrhosis [11]. This study showed that the crude aqueous leaf extract of Ficus benjamina has a potential hepatoprotective property on ethanol-induced hepatotoxicity in mice. Such property can be attributed through several studies, which have shown that F. benjamina have antitumor, anti-inflammatory, antioxidant, and cytotoxic

activities which could negate the deleterious effect of alcohol in the liver. According to Sirisha et al. (2010), Ficus species are rich in polyphenolic compounds and flavonoids, which are responsible for its antioxidant properties that help prevent and treat oxidative stress related to hepatic diseases. F. benjamina contains naringenin, quercetin, and caffeic acid, which are both antioxidants and anti-inflammatory agents [12]. Caffeic Acid or 3,4-dihydrocinnamic acid contains a catechol with an α, β-unsaturated carboxylic acid chain that has a hepatoprotective property, which is achieved through the inhibition of 5-lipooxygenase by uncompetitive inhibition and by decreasing the chemiluminiscence of phorbol ester-stimulated neutrophil and of a cell-free superoxide superoxide generating system [13]. Quercetin, on the other hand, prevents the decrease in copper/zinc superoxide dismutase activity, which is an important antioxidant during acute inflammation. It also lessens inflammation in the liver by down regulating the nuclear factor-kappa β, tumor necrosis factor-α, transforming growth factor-1 and cyclooxygenase. Treatment with quercetin also significantly increases antioxidants expression in injured livers [14]. In a study of Jayaraman et al. (2012), it was shown that naringenin has anti-inflammatory effects in ethanol-induced hepatotoxicity in rats by decreasing levels of serum aspartate and alanine transaminases, iron, ferritin, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), nuclear factor-kappa B (NF-κB), cyclooxygenase-2 (COX-2), macrophage inflammatory protein 2 (MIP-2) and CD14. Recent studies have shown that these compounds are also responsible for the hepatoprotective property of this plant [15].

5 Conclusion

Crude aqueous leaf extract of Ficus benjamina has hepatoprotective effect on ethanol-induced hepatotoxicity in mice similar to Silymarin. This study also validated the use of ethanol as model for hepatotoxicity study in mice. F. benjamina, as an ornamental plant, may be a source of phytochemical with potential pharmaceutical and functional activities. However, as a non-food source, toxicity study should be done for this particular plant and its ethnobotanical characterization should be further studied.

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