Research Article

Effect of *Vitis vinifera* L Seed Extract on Hepatic Marker Enzymes and Oxidative Stress against Acetaminophen Induced Hepatotoxicity in Rats

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ABSTRACT

To investigate the hepatoprotective potential of ethanolinc grape seed extract on acetaminophen (APAP) induced liver toxicity in wistar rats. Silymarin was used as reference hepatoprotective agent. Liver injury was induced by APAP (2 gm/kg body weight, bw) on the 1st day of experiment. *Vitis vinifera* (*V. vinifera*)L seed extract (200 and 400 mg/kg bw) and silymarin (100 mg/ kg bw) was administered orally daily for next 6 days. The animals were sacrificed on 7th day and the hepatoprotective activity was assessed by using various biochemical parameters. In APAP treated rats, the aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin levels were significantly increased, while lipid profile levels were altered. Treatment with APAP significantly elevated hepatic malondialdehyde (MDA) but reduced hepatic superoxide dismutase (SOD), Catalase, Glutathione peroxidase (GPx) levels. The *Vitis vinifera* L seed extract and silymarin administration prevented the toxic effect of APAP on the AST, ALT, ALP, total bilirubin and lipid profile and elevated hepatic MDA and decreased hepatic SOD, Catalase, GPx levels. The present study concludes that *Vitis vinifera* L seed extract exhibits significant hepatoprotective activity, which may be due to presence of bioactive compounds such as flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidins and resveratrol.

Keywords: *Vitis vinifera* L, Acetaminophen, Hepatic markers, Lipid profile, Lipid peroxide.

INTRODUCTION

The liver is the chief organ for metabolism of carbohydrates, lipids, proteins and detoxification of drugs and toxins. However, drugs affect the liver more commonly than any other organ and place the liver at a great risk of toxic damage[1]. After absorption of drugs by the intestine, it reaches the liver via the portal system. Liver disease remains as one of the serious health problems throughout the world. More than 350 million people were affected with chronic hepatic infections worldwide and in India, above 20,000 deaths were reported every year due to liver disorders. Hepatocellular carcinoma is one of the most common tumors in the world with over 250,000 new cases each year[2].

Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) is a widely used analgesic and antipyretic drug[3-4]. Although considered safe at therapeutic doses, it could induce hepatotoxicity higher doses and even acute liver failure (ALF)[5]. APAP can be metabolized by cytochrome P450 enzymes (CYPs) to N-acetyl-P-benzoquinoneimine (NAPQI)[6]. At overdoses of APAP, a large number of NAPQIs are generated, which can diminish the reduced glutathione (GSH) and then bind to mitochondrial proteins, which leads to mitochondrial dysfunction and oxidative stress[7], this results in hepatocellular damage. In this process, APAP can increase the level of malondialdehyde (MDA)[8] and NAPQI is capable of lowering GSH/oxidized glutathione (GSSG) ratio by oxidizing the thiol group of GSH. Oxidative stress plays a pivotal role in the hepatic damage induced by APAP[9]. Antioxidant function against oxidative stress are probably the most invoked mechanisms of protection by natural products. In recent days, natural products with antioxidant capacity have been used for centuries all over the world. Many of the natural products are claimed to assist in healthy lifestyle. Medicinally, natural

products have made a significant contribution for the treatment of hepato toxicity[10]. Grape, *Vitis vinifera* (Family: Vitaceae) is one of the most popular and the world's largest fruit crop. In Ayurveda, the aerial parts of *Vitis vinifera* have been widely used for the treatment of variety of common and stress related disorders. Grape contains a variety of active components including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidins and resveratrol. The major medicinal properties of grape and its constituents are lowering of low-density lipoprotein, reduction of cardiovascular diseases, anticarcinogenic, immunomodulatory, antidiabetes, anti-obesity, and anti-aging[11-13]. Keeping the folkloric claims and reports in view, the present study attempted to assess the possible hepatoprotective potential of the crude ethanolic seed extract of *Vitis vinifera* in acetaminophen -induced hepatotoxicity in rats.

MATERIAL AND METHODS

Preparation of extract

The ripe fruits of *Vitis vinifera* were collected from Tirupathi, Andhra Pradesh, India in the month of October 2012 and the seeds were separated from the pulp and shade dried. The dried powdered seed material of *Vitis vinifera* (2 kg) was extracted with 5L ethanol (95%) shaking occasionally at room temperature for 3 days, then covered by a piece of aluminum foil and kept in refrigerator. After filtration, the solvent was concentrated on rotary evaporator (Rotavapor® R-210) at 40-50ºC under reduced pressure. The 5g of crude organic extract was obtained.

Chemical and drugs

The acetaminophen was purchased from SmithKline Pharma, Mumbai, India. Silymarin was obtained from Micro Labs Limited, Baddi, Himachal Pradesh, India. Reduced glutathione (GSH) and glutathione were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Thiobarbituric acid was purchased from E-Merck, India. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin estimated kits were procured from Span Diagnostics, Surat, India. All other chemicals and reagents used were of analytical grade.

Animals

Male albino wistar rats (180 ± 20 g bw) were obtained from Sri Venkateswara traders, Bangalore, India and were maintained under a constant 12 h light and dark cycle at 21-23°C. The animals were fed with commercial pellet diet (Ratan Brothers, Hyderabad, India) and tap water was provided *ad libitum* throughout the experiment period. Animals were kept for seven days in laboratory for habituation. The experiments were carried out in accordance with the guidelines of National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. This study was approved by the institutional animal ethical committee (10/(i)/a/CPCSEA/IAEC/SVU).

Experimental induction of hepatotoxicity

Hepatotoxicity was induced in animals by oral administration of acetaminophen (2 g/kg bw) in a freshly prepared 0.9% of normal saline as a single dose in the 1st day of experiment. The animals were randomly divided into 5 groups of six rats each as mentioned below. *V. vinifera* seeds extract and silymarin were suspended in 2 % of gum acacia. *V. vinifera* seeds extract and silymarin were administered orally once in a day for 6 days.

Group 1: Normal control rats (2% gum acacia).
Group 2: Acetaminophen (2 mg/kg bw)
Group 3: Acetaminophen + *V. vinifera* seeds extract (200 mg/kg bw)
Group 4: Acetaminophen + *V. vinifera* seeds extract (400 mg/kg bw)
Group 5: Acetaminophen + Silymarin (100 mg/kg bw)

On 7th day morning animals were sacrificed by cervical dislocation. The blood collected into clean test tubes and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min. The liver was immediately removed and cleaned in ice-cold saline to remove adjacent tissues and homogenized in 0.1 M Tris-HCl buffer (pH7.0). The homogenate was centrifuged at 2000 rpm for 10 min at 4ºC in a cold centrifuge. The supernatants were separated and used for biochemical analysis.

Biochemical estimations

The serum levels of AST, ALT, ALP and total bilirubin were assayed by using Span Diagnostics, Surat, India kits. Lipid peroxidation (LPx) was estimated in terms of malondialdehyde (MDA) content and determined by using the thiobarbituric acid (TBA) by the method of Hiroshi et al.,[14]. The activity was expressed as µmol of malondialdehyde formed/g wet weight of tissue. Superoxide dismutase (SOD) (EC 1.15.1.1) was assayed for its ability to inhibit the auto-oxidation of epinephrine in alkaline medium[15]. The SOD activity levels were expressed in units per mg protein per min.

Catalase (EC 1.11.1.6) was assayed by the method of Maehly & Chance[16] by determining the decrease in the concentration of hydrogen peroxide (H_2O_2). The activity of enzyme was expressed in µmol of hydrogen peroxide (H_2O_2) metabolized/mg protein/min. Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al.,[17]. The activity was expressed as µMol of GSH consumed/ mg protein/min. The protein concentration was determined using bovine serum albumin as the standard[18] .

Data and Statistical analysis

All analyses were performed with statistical package for social sciences (SPSS) 13.0 for Windows (SPSS, USA). The data were expressed as mean with S.D. The significance was determined by applying student's paired 't' test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Results presented in Table 1 indicate that the levels of serum enzymes namely AST, ALT, ALP and total bilirubin levels were significantly ($p<0.01$) increased in acetaminophen treated rats when compared with normal rats. However, treatment of rats with *V. vinifera* and silymarin significantly decreased ($p<0.01$) serum enzymes like AST, ALT, ALP and total bilirubin levels when compared to acetaminophen treated rats. The lipid peroxidation levels were significantly ($p<0.01$) increased in the liver of acetaminophen treated rats when compared to that of normal control rats. However, *V. vinifera* and silymarin treated rats showed significant ($p<0.01$) declined in lipid peroxidation levels when compared to acetaminophen treated rats. The antioxidant enzymes like SOD, catalase, GPx levels were significantly ($p<0.01$) decreased in liver of acetaminophen treated rats. Furthermore, *V. vinifera* and silymarin treated rats showed normalization of SOD, catalase, GPx levels when compared to acetaminophen treated rats (Table 2).

DISCUSSION

Acetaminophen induced hepatotoxicity has been previously documented by a number of studies. Acetaminophen is a well known antipyretic and analgesic agent. In excess doses, it causes severe hepatic damage in humans and experimental animals. It is used commonly as a model for hepatotoxicity. Formation of a reactive metabolite viz. N-acetyl-p-benzoquinoneimine (NAPQI) by the action of cytochrome P-450 enzyme system contributes to the hepatotoxicity of the acetaminophen[20]. This generates the reactive oxygen species, thereby enhances oxidative stress which leads to liver injury and hepatocellular death[21]. When there is damage to the liver cell membrane, the cytosolic enzymes are leaked into the blood stream[22]. Therefore, the elevation of these cytosolic enzymes in the blood stream is a needful quantitative marker for the extent of hepatic damage. The elevated levels of the AST, ALT, ALP and total bilirubin levels in the rats administered with acetaminophen indicate the hepatocellular damage and alterations in the membrane permeability. Our reports on these elevated levels during acetaminophen induced hepatic damage are in accordance with the previous reports[23]. Treatment with the *V. vinifera* attenuated the elevated levels of ASP, ALT, ALP and total bilirubin levels.

However, liver has a number of mechanisms to keep itself away from the toxic effects of free radicals generated by acetaminophen. Reduction in the activity of SOD causes a rise in the levels of superoxide radical production. Catalase is responsible for detoxification of H_2O_2, therefore decreased catalase activity reflects ineffective scavenging of H_2O_2. GPx removes peroxyl radicals from various peroxides including H_2O_2 (Pirinççioğlu et al., 2012). The present study revealed a significant decrease in lipid peroxidation levels with significant increase in SOD, catalase and GPx activity levels in liver of *V. vinifera* treated rats. These results suggest the protective role of *V. vinifera* in oxidative impairment of liver in acetaminophen treated rats [23,24].

Earlier studies demonstrated that *V. vinifera* extract at an oral dose of 200 mg/kg exhibited a significant protective effect by lowering the serum levels of SGPT, SGOT, alkaline phosphatase and total bilirubin[12]. Our results are consistent with earlier studies, which strongly suggest that *V. vinifera* may protect the structural integrity of hepatocytes and prevent the leakage of cytosolic enzymes into bloodstream. Additionally, *V. vinifera* showed scavenging activity, suggesting that it could scavenge the free radicals generated during acetaminophen metabolism. The antioxidant property of *V. vinifera* may be due to flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidins and resveratrol[25]. These compounds quench ROS and regenerate membrane-bound antioxidants levels during aadministration of *V. vinifera* at different dose levels.

CONCLUSIONS

The present study concludes that the ethanolic seed extract of *V. vinifera* shows a protective

effect against paracetamol induced hepatotoxicity in experimental rats. However, further investigation is in process on the seeds extract to identify the active constituents' responsibility for hepatoprotection.

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Table 1: Effect of *V. vinifera* ethanolic seeds extract and silymarin on AST, ALT, ALP and total bilirubin on acetaminophen induced hepatotoxicity in wistar rats

Groups	AST	ALT	ALP	Total bilirubin
Normal rats	23.2 ± 1.53	22.6 ± 5.72	46.3 ± 3.4	0.45 ± 0.03
PCM control	145.6$^{\#}$ ± 7.25	115.2$^{\#}$ ± 1.4	280.3$^{\#}$ ± 9.4	4.52$^{\#}$ ± 0.04
PCM + *V. vinifera* (200mg/kg bw)	107.6* ± 2.42	54.8* ± 2.7	193.6*± 5.2	2.62* ± 0.03
PCM + *V. vinifera* (400 mg/kg bw)	98.1* ± 2.58	43.4* ± 2.2	180.4* ± 3.5	3.53* ± 0.01
PCM + silymarin (100 mg/kgbw)	85.3*± 4.21	35.8* ± 2.8	168.4* ± 5.7	3.25* ± 0.02

Values are expressed as Mean ± SD of 6 individuals.
ns= non significant; PCM= paracetamol
#P<0.01, compared with normal control
*P<0.01, compared with PCM control

Table 2: Effect of *V. vinifera* ethanolic seeds extract and silymarin on lipid peroxidation, SOD, Catalase and GPx levels on acetaminophen induced hepatotoxicity in wistar rats

Groups	Normal rats	PCM control	PCM + *V. vinifera* (200mg/kg bw)	PCM + *V. vinifera* (400 mg/kg bw)	PCM + silymarin (100 mg/kgbw)
Lipid peroxidation (µ moles of malondialdehyde formed/gram wet weight of tissue)	4.15 ± 1.35	24.21$^{\#}$ ± 2.54	18.54* ± 2.35	12.06* ± 2.68	11.45* ± 3.14
Superoxide dismutase (units/mg protein/min)	1.68 ± 0.05	0.85$^{\#}$ ± 0.06	1.25* ± 0.14	1.43* ± 0.24	1.46* ± 0.16
Catalase (H_2O_2 metabolized/mg protein/min)	0.64 ± 0.05	0.36$^{\#}$ ± 0.06	0.45*± 0.04	0.52* ± 0.07	0.56* ± 0.04
Glutathione peroxidase (µMol of GSH consumed/min/mg protein)	1.62 ± 0.08	1.15$^{\#}$ ± 0.06	1.35* ± 0.02	1.46* ± 0.04	1.54* ± 0.07

Values are expressed as Mean ± SD of 6 individuals.
ns= non significant; PCM= paracetamol
#P<0.01, compared with normal control
*P<0.01, compared with PCM control

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