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Protective Effect of *Hordeum vulgare* Linn. on Acetaminophen-Induced Liver Damage

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ABSTRACT

The objective of the present study has been to evaluate the hepatoprotective activity of methanolic extract of *Hordeum vulgare* Linn. (MEHV) seeds against acetaminophen-induced liver damage in rats. Acetaminophen-induced liver damage was produced by the treatment of acetaminophen (3g/kg/d, p.o.) for three days. Other groups of rats were pretreated with two doses of MEHV seeds (300 and 500 mg/kg/d, p.o) and silymarin (200 mg/kg/d, p.o) 30 min prior to acetaminophen ingestion. Liver damage was evidenced by elevated levels of biochemical parameters such as serum glutamate oxaloacetic transaminase and glutamate pyruvic transaminase (SGOT and SGPT), alkaline phosphatase (ALP), total and direct bilirubin (TBL and DBL), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), malondialdehyde (MDA), decreased level of total protein (TP) and reduced glutathione (GSH) along with increased histopathological scores in the APAP control. Pretreatments with MEHV seeds produced significant reversal in the above biochemical parameters and reduced histopathological scores of fatty degeneration, necrosis with significant evidence of regeneration. The results of this study indicate that pretreatment with MEHV seeds possessed the significant hepatoprotective activity. In conclusion, the possible mechanism of hepatoprotective action of methanolic extracts of Hordeum vulgare seeds may be due to its antioxidant activity as indicated by protection against increased lipid peroxidation and maintained glutathione contents.

Key words: Antioxidant, APAP, hepatoprotective activity, *Hordeum vulgare*, phenolic compounds

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INTRODUCTION

Hordeum vulgare Linn. (Poaceae), which is commonly known as barley, is an erect annual herb, 50 to 100 cm height, cultivated in the plains as well as in the hilly region of Himalaya up to an altitude of 4000 m.[1] It is locally called Jav and its seeds are used by traditional medical practitioners in the treatment of many diseases including liver diseases.[2-3]

Phenolic compounds are presumed to be responsible for beneficial effects derived from the consumption of whole grains, fruits, and vegetables. The antioxidant properties of phenolic compounds in grains have been associated with the health benefits attributed to these crops and the value-added products derived from them. Antioxidants may play an important role in the prevention of chronic diseases by arresting oxidative damage caused by reactive oxygen species (ROS) to vital biomolecules such as DNA, lipids, and proteins.[4] H. vulgare L., barley is rich in a wide range of antioxidant compounds such as phenolic acid derivatives, proanthocyanidins, quinones, and flavonoids.[5]

There have been some studies on the antioxidant activity and phenolic content of barley.[5-9] As the anti-oxidant and hepatoprotective activities of certain phenolic compounds from plant origin have already been established, we can speculate that these constituents may be responsible for the observed protective effects.[10-11] Therefore, an attempt was made to evaluate the hepatoprotective activity of methanolic extract of *H. vulgare* Linn (MEHV) seeds against acetaminophen-induced liver damage in Wistar albino rats.

MATERIALS AND METHODS

Chemicals

Acetaminophen (APAP) was purchased from S. D. Fine Chem. Ltd, Mumbai. Silymarin was obtained as a gift sample from Micro labs, Bangalore, India. Serum glutamate oxaloacetic transaminase and glutamate pyruvic transaminase (SGOT and SGPT), alkaline phosphatase (ALP), Bilirubin kits were procured from Span Diagnostics, Surat, India. Lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT) kits were procured from Coral Clinical Systems, Goa, India. All other chemicals and reagents used were of analytical grade.

Plant materials and extraction

Seeds of *H. vulgare* were purchased from a commercial supplier, identified and authenticated by Dr. A. S. Reddy, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India where a voucher specimen (No. MP-3: 28/7/2007) was kept for the future reference. The seeds (unhulled) were dried at room temperature and mechanically powdered to obtain a coarse powder. Methanolic extract was prepared by macerating a powder with methanol/water (70/30, v/v) for 48 h with constant stirring. It was filtered, and the filtrate was evaporated under vacuum using a rotary evaporator to obtain a light brown crystalline powder (10 ± 0.12%w/w yield). The same procedure was repeated thrice in order to get the reproducibility of yield of extract. The dry methanolic extract was stored in a cool and dry place.

Phytochemical analysis

Preliminary phytochemical studies of the extract were performed for the detection of various phytoconstituents viz., alkaloids, flavonoids, saponins, glycosides, phenols, steroids, tannins and terpenoids according to standard procedures.[12-13]

Determination of total phenolic content (TPC)

The total phenolic content of the extract was estimated according to a modified Folin-Ciocalteu method.[7] Observing a sequence specified here, the following were introduced into a test tube: 1 ml of diluted sample or standard solution, 4 ml of Folin-Ciocalteu working solution, 5 ml of sodium carbonate (7.5%, w/v). This solution was agitated and left to stand for 2 h for the reaction to take place and stabilize. The absorbance was recorded at 740 nm. The calibration curve was performed with gallic acid, and the results are expressed as milligrams of gallic acid equivalents (GAE) per grams of dry weight of seeds.

Experimental animals

Wistar albino rats of either sex (200-220 g) were obtained from the animal house, Anand pharmacy college (APC), Anand, India and housed in standard condition of temperature (22 ± 2°C), relative humidity (55 ± 5 %) and light and dark cycles (12 h/12 h) were used. Rats were fed with standard laboratory food and water ad libitum. Animal studies were approved by Institutional Animal Ethics Committee (Protocol No. 7005 dated 07/08/07) and conducted according to the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity studies

Acute toxicity study was conducted for MEHV seeds by stair case method as per OECD guidelines-425, 2001.[14] There was no lethality up to a dose of 5000 mg/kg. One tenth of the maximum, that is, 500 as well as 300 mg/kg doses were selected for the evaluation.[15-16]

Hepatoprotective activity

Wistar rats were randomly divided into five groups where each group contains six rats: Group I (Normal control) was received distilled water, p.o. and Group II (APAP control) was treated with APAP (3g/kg/d, p.o.) for three days. Group III (Test-1) and Group IV (Test-2) were pretreated with MEHV at a dose of 300, 500 mg/kg, and Group V (Standard control) silymarin at a dose 200 mg/kg, p.o., respectively 30 min prior to acetaminophen ingestion for three days.[16]

At the end of treatment, blood was obtained from all animals by puncturing retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot for

45 min at room temperature. Serum was separated using Plasto Craft's refrigerated ultracentrifuge [Rota 4R-V/FA] at 2500 rpm at 4ºC for 15 min and utilized for the estimation of various biochemical parameters such as SGOT and SGPT;[17] ALP;[18] TBL and DBL;[19]LDH;[20] GGT[21] and TP.[22]

After collection of blood samples, the rats were sacrificed by ether anesthesia and abdomen was cut open to remove the liver. Liver sections of 5 µm were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 h, then embedded in paraffin and stained using haematoxylin-eosin (H and E) dye, finally mounted in diphenylxylene.[23] The histological sections were evaluated by a pathologist unaware of the experiments being performed under a light microscope. The histological scoring of rat liver was determined by examining each specimen for degeneration, necrosis, regeneration and score given depends upon the severity of the findings: none = score 0; mild = score 1; moderate = score 2; severe = score 3.[24]

Remaining portion of livers was rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation.[25] A part of homogenate after precipitating proteins with Trichloro acetic acid (TCA) was used for the estimation of GSH.[26]

Statistical analysis

The experimental results were expressed as mean ± SEM for six animals in each group. The biochemical parameters were analyzed statistically using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (DMCT). Liver histopathological scores were analyzed by Kruskal - Wallis test followed by Mann- Whitney U test. P values <0.05 were considered as significant.[27-28]

RESULTS

The results of preliminary phytochemical analysis of MEHV seeds revealed the presence of phytoconstituents such as phenolic compounds, flavonoids, saponins, terpenoids and glycosides. According to previous study,[7] 70% aqueous methanol was used to extract various phenolic compounds from seeds. Total phenolic content of methanolic extract was found to be 18.3 mg GAE ± 0.2 /g of dry weight of seeds. The extract did not produce any toxic symptoms of mortality up to the dose level of 5000 mg/kg body weight in rats, and hence the drugs were considered to be safe for further pharmacological screening.

The increased levels of serum biochemical parameters such as SGOT, SGPT, ALP, TBL, DBL, LDH, GGT, MDA and decreased levels of TP and GSH were found in APAP control. Pretreatments with Test-1 and Test-2 caused significant reversal in above parameters [Table 1].

Histopathological examination of the normal control showed a normal architecture of the liver with distinct hepatic cells, sinusoidal spaces and central vein [Figure 1A]. APAP control increased the scores of degeneration and necrosis [Figure 1B]. Pretreatments with Test-1, Test-2 and standard control preserved the normal structure of liver [Figure 1C, D and E] by significantly reducing the scores of degeneration, necrosis with evidence of significant regeneration [Table 2].

DISCUSSION

Protection against APAP-induced liver damage has been taken as a test for potential hepatoprotective agent by several investigators.[29-30] The hepatotoxicity of APAP has been reported to be caused by the formation of N-acetyl-p-benzoquinoneimine (NAPQI), which is a toxic metabolite, and accompanied prominent increase of SGOT, SGPT and

Table 1: Effects of methanolic extract of *H. vulgare* seeds on various biochemical parameters in APAP induced liver damage

Biochemical parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
SGOT (IU/L)	33.50 ± 4.69	170.42 ± 6.38##	88.17 ± 2.92*	80.26 ± 5.03*	48.58 ± 1.47*
SGPT (IU/L)	28.17 ± 2.17	120.00 ± 9.56#	65.83 ± 4.00*	56.33 ± 2.33*	39.00 ± 1.19*
ALP (KAU/dl)	7.18 ± 0. 71	59.17 ± 0.76##	31.33 ± 3.12*	17.51 ± 4.99*	11.03 ± 0.04*
TBL (mg/dl)	0.36 ± 0.04	6.57 ± 0.81##	1.70 ± 0.09*	1.54 ± 0.09*	1.30 ± 0.10*
DBL (mg/dl)	0.14 ± 0.02	11.46 ± 3.19#	2.04 ± 0.22*	1.54 ± 0.23*	1.23 ± 0.10*
LDH (U/L)	426.07 ± 12.63	1766.22 ± 12.94##	866.14 ± 18.51*	754.32 ± 21.03*	471.23 ± 6.51*
GGT (U/L)	39.08 ± 2.99	89.82 ± 2.29##	56.20 ± 2.04*	50.93 ± 1.31*	37.38 ± 2.21*
TP (mg/ml)	7.69 ± 0.77	4.37 ± 0.04#	8.47 ± 0.33*	9.32 ± 0.12*	10.19 ± 0.03*
MDA (µg/mg protein)	0.26 ± 0.02	1.68 ± 0.01##	0.48 ± 0.03*	0.46 ± 0.007*	0.43 ± 0.003*
GSH (µg/mg protein)	12.19 ± 1.60	8.40 ± 0.14##	11.73 ± 0.30*	12.82 ± 0.77*	11.98 ± 0.41*

Values are expressed as mean ± SEM; n=6 rats in each group; #P<0.05 and ##P<0.001 are considered significant when compared with group I using ANOVA; *P<0.05 is considered significant when compared with group II using ANOVA followed by DMCT.

Table 2: Effect of methanolic extract of *H. vulgare* seeds on liver histopathology score in APAP induced liver damage

Histopatholog-ical parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
Degeneration	0	2.8 ± 0.27##	1.33 ± 0.21**	0.67 ± 0.21**	0.50 ± 0.22**
Necrosis	0	2.67 ± 0.21##	1.33 ± 0.17*	0.83 ± 0.17**	0.50 ± 0.22**
Regeneration	0	0	1.0 ± 0.0**	1.33 ± 0.21**	1.67 ± 0.21**

Values are expressed as mean ± SEM; n=6 rats in each group; Kruskal-Wallis revealed significant difference between all groups. Mann-Whitney U test: - # P<0.05 and # # P<0.01 are considered significant when group II compared with group I; *P<0.05 and ** P<0.01 are considered significant when group II compared with group III, group IV and group V.

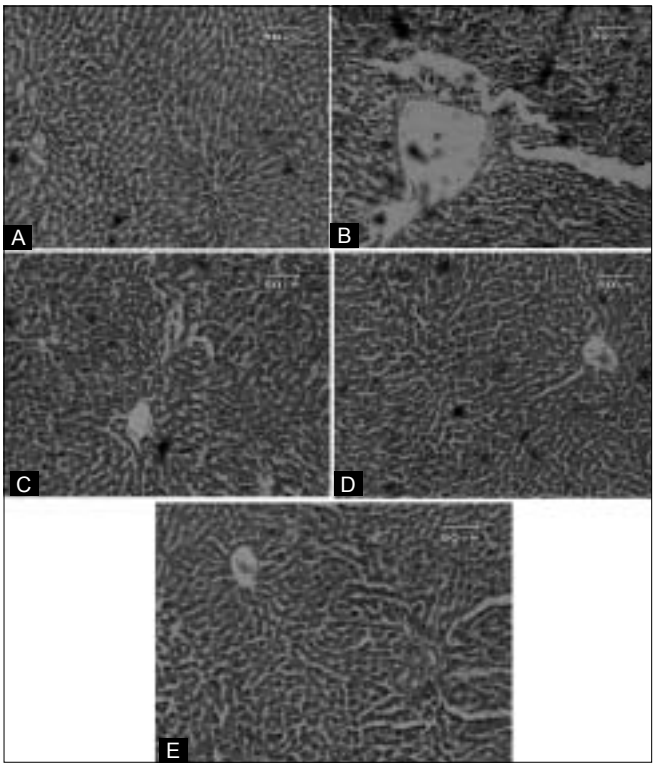

Figure 1: Histopathological changes occurred in the experimental groups during APAP intoxication and prevention by the pretreatment with Hordeum vulgare seeds methanolic extract (Haematoxylin and eosin, 200X). (A) Normal control, (B) APAP control, (C) Test -1 (300 mg/kg) + APAP, (D) Test -2 (500 mg/kg) + APAP, (E) Silymarin (200 mg/kg) + APAP.

ALP levels.[31-32] According to Table 1, liver damages may be repaired by reducing the SGOT, SGPT and ALP levels. Our results demonstrated that MEHV had the hepatoprotective effect to prevent APAP-induced liver damage. In addition, MEHV also preserved the normal structure of liver by significantly reducing the scores of degeneration, necrosis with evidence of significant regeneration. This confirms the hepatoprotective activity of the MEHV seeds against APAP-induced liver damage in rats.

Regarding the metabolism of NAPQI, small amount of NAPQI is rapidly metabolized by the glutathione (GSH), but the exceeding amount of NAPQI produces hepatic necrosis, which increases utilization of GSH and depletes GSH storage in the liver.[33-35] Moreover, NAPQI can also increase the formation of reactive oxygen species (ROS) including the superoxide anion, hydroxyl radical, and hydrogen peroxide. Excess levels of the ROS, which leads to enhancement of lipid peroxidation and reduction of the antioxidant enzymes, attack biological molecules such as DNA, protein, and phospholipids.[36-38] This mechanism has been suggested to play a role in the development of oxidative stress and injury in APAP-induced liver damage.[39-40] In the present study, the decreased level of GSH has been associated with elevated levels of MDA, end products of LPO in APAP. The increase in MDA level in liver suggests enhanced LPO leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radical. Table 1 shows that the pretreatment with Test-1, Test-2 enhanced the GSH and diminished the lipid peroxidation (MDA) levels. These findings demonstrated that the MEHV possessed significant antioxidant activities that provide protection from the APAP-induced liver damage in rat. This fact is consistent with those of the previous reports.[5-9]

Hordeum vulgare L., Barley is rich in a wide range of antioxidant compounds such as phenolic acid derivatives, proanthocyanidins, quinones and flavonoids.[5] Phenolic compounds were considered as a major group of compounds that contributed to the antioxidant activity of cereal.[41] Significant amounts of total phenolics were detected in methanolic extract using the Folin-Ciocalteu phenol reagent method which was higher than those reported by Dvorakova *et al.* This might be due to the difference in the extraction methods used in this study. These total phenolic compounds present in extract might be responsible for the protective action upon APAP-induced liver damage.

The result of total phenolic contents, serum biochemical parameter, level of hepatic lipid peroxides, hepatic glutathione and histopathological studies in the pre-treatment groups together support the highly potent hepatoprotective activity of methanolic extracts of *Hordeum vulgare* seeds and also supports the traditional use of *H. vulgare* for the treatment of hepatic disorders.

In conclusion, the possible mechanism of hepatoprotective

action of methanolic extracts of *Hordeum vulgare* seeds may be due to its antioxidant activity as indicated by protection against increased lipid peroxidation and maintained glutathione contents. Rest of the biochemical and histopathological parameters studied indicate the status of structural and functional integrity of the cells and provide further support to the suggestive mechanism of action.

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