HEPATOPROTECTIVE ACTIVITY OF *HORDEUM VULGARE* LINN. SEEDS AGAINST ETHANOL-INDUCED LIVER DAMAGE IN RATS

Purvi A. Shah*, Mihir Y. Parmar, Vaishali T. Thakkar, Tejal R. Gandhi

Anand Pharmacy College, Shree Ramkrishna Seva Mandal Campus, Opp. Town Hall, Anand, Gujarat.

Summary

Alcohol toxicity is one of the world's major health problems. Herbal alternatives are one of the best ways to minimize these disease conditions. In the present study, methanolic extract of *Hordeum vulgare* Linn. (Family: Poaceae) seeds was investigated for the hepatoprotective activity against ethanol-induced liver damage in rats. Ethanol-induced liver damage in rats was induced by oral administration of 20% ethanol (3.76 g/kg/d) for 18 days. Other groups of rats were pretreated with methanolic extracts of *Hordeum vulgare* seeds (300 and 500 mg/kg, p.o.) or silymarin (200 mg/kg, p.o.) 30 min prior to ethanol ingestion. The degree of protection was measured by various liver parameters such as functional (thiopentone-induced sleeping time), physical (liver weight and volume), serum biochemical parameters like serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase (SGOT & SGPT), alkaline phosphatase (ALP), total and direct bilirubin (TBL & DBL), total cholesterol (TC), triglyceride (TG) and total protein (TP) along with changes in histological parameters. Ethanol produced significant changes in various liver parameters such as functional (thiopentone-induced sleeping time) and physical (increased liver weight and volume). It also increased above biochemical parameters and decreased total protein (TP) along with changes in histological parameters (damage to hepatocytes). Treatments with methanolic extract of *H. vulgare* (300 and 500 mg/kg/d, p.o. for 18 d) and silymarin significantly prevented the functional, physical, biochemical and histological changes induced by ethanol, indicating the recovery of hepatic cells. These results demonstrate that methanolic extract of *H. vulgare* seeds possessed the hepatoprotective activity as evidenced from the functional, physical, biochemical and histological parameters.

Key Words: *Hordeum vulgare*, Hepatoprotective activity, Ethanol, Silymarin.

***Corresponding author: Mrs. Purvi A. Shah**
Anand Pharmacy College, Opposite Town hall, Anand -388 001
Gujarat, India Telefax number: 91-02692-250020
E- mail: purvi2210@yahoo.co.in

Introduction

Alcohol toxicity is one of the world's major health problems as the significant numbers of peoples are affected due to several fatal diseases caused by alcohol. Herbal alternatives are one of the best ways to minimize these disease conditions. For this, we have chosen ethanol-induced toxicity in rat model to study effect of ethanol on liver [1].

As a herbal alternative, we have used *Hordeum vulgare* Linn. seeds (Commonly known as barley, Family: Poaceae), is an erect annual herb, 50 to 100 cm high, cultivated in the plains as well as in the hilly region of Himalaya, up to an altitude of 4000 m, in Indo- Gangetic area and Madhya Pradesh. It is locally called Jav and its seed used by traditional medical practitioners in the treatment of many diseases including liver diseases. Seeds are useful in vitiated conditions of kapha and pitta, asthma, fever, bronchiotitis, urocystitis, urethritis, gastric disorders, ulcers and anemia etc [2]. Different parts of plant have been reported to possess a numerous pharmacological activities such as antiulcer [3], antifungal [4], antidiabetic [5], antimutagenic [6], hypocholesterolemic [7] and in-vitro antioxidant activity [8]. However there is no scientific evidence to support that the seeds can be used in the prevention or treatment of ethanol-induced liver damage. Therefore, the present study was carried out to evaluate the hepatoprotective activity of methanolic extract of *H. vulgare* seeds.

Materials and Methods

Drugs and Chemicals:

Ethanol was purchased from Baroda Chemical Industries Ltd, Dabhoi. Silymarin was obtained as a gift sample from Micro labs, Bangalore, India and used as such. SGOT, SGPT, ALP, Bilirubin and TC kits were procured from Span Diagnostics, Surat, India. TG kits were procured from Reckon diagnostic Pvt. Ltd, Baroda, India. All other chemicals and reagents used were of analytical grade.

Plant material and extraction:

H. vulgare seeds 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 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 hrs with constant stirring. It was filtered; filtrate was evaporated under vacuum using a rotary evaporator to obtain a light brown crystalline powder (10 ± 0.12%w/w yield). The dry methanolic extract was stored in cool and dry place, which further used for the evaluation.

Phytochemical screening:

A preliminary phytochemical screening of methanolic extract of *H. vulgare* seeds was carried out [9].

Animals:

Wistar albino rats of either sex (200-220 g) 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 methanolic extract of *H. vulgare* seeds by stair case method following OECD guidelines-425, 2001 [10]. There was no lethality up to a dose of 5000 mg/kg. One tenth of the maximum dose of the extract i.e. 500 as well as 300 mg/kg was selected for the evaluation of the hepatoprotective activity [11].

Experimental protocols:
Animals were randomly divided into five groups six of each. All animals except normal control group were intoxicated with 20% ethanol (3.76 g/kg/d, p.o) for 18 days. **Group I (Normal control)** received only distilled water and **Group II (Ethanol control)** received 20% ethanol (3.76 g/kg/d, p.o) for 18 days. **Group III (Test-1)** and **Group IV (Test-2)** pretreated with 300 and 500mg/kg, p.o. methanolic extract of *H. vulgare* seeds respectively while **Group V (Positive control)** pretreated with 200 mg/kg, p.o. silymarin 30 min prior to ethanol ingestion [12]. On day 19, thiopentone sodium (40 mg/kg, i.p) was injected and the sleep time recorded in all the animals.

After complete recovery from thiopentone sodium effect, blood was collected from retro-orbital plexus of overnight fasted rats. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 4 ºC for 15 min and used for the estimation of various biochemical parameters such as SGOT and SGPT [13], ALP [14], TBL and DBL [15], TC [16] TG [17] and TP [18].

After collection of blood samples, the rats were sacrificed by ether anesthesia and their livers were excised, rinsed in ice-cold normal saline and the wet-weights and volumes were determined. Randomly selected portions of liver from each group of animals were utilized for the histological study. In brief, 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 & E) dye, finally mounted in diphenylxylene [19]. The sections were examined under a light microscope for the histological assessment, which was done by using a method of scoring of the structural changes described by National Institute of health, Maryland, USA [20].

Statistical Analysis:
The experimental results were expressed as mean ± SEM for six animals in each group. The physical, functional and biochemical parameters were analyzed statistically using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (DMCT). Liver histological data were analyzed by Kruskal- Wallis test followed by Mann- Whitney U test. P values <0.05 were considered as significant [21].

Results

Preliminary phytochemical screening of methanolic extract of *H. vulgare* seeds revealed the presence of phenolic compounds (flavonoids), terpenoids, glycosides and saponins. The methanolic extract of *H. vulgare* seeds was found to be nontoxic up to a dose of 5000 mg/kg. A significant increased in functional parameter such as thiopentone-induced sleep time and physical parameters such as weight and volumes of wet-liver were found in ethanol control as compared to normal control. Treatments with Test-1, Test-2 and positive control caused significant decreased in functional and physical parameters as compared to ethanol control (Table 1). A significant increased in biochemical parameters such as SGOT, SGPT, ALP, TBL, DBL, TC, TG and decreased level of TP were found in ethanol control as compared to normal control. Treatments with Test-1, Test-2 and positive control caused significant reversal in above biochemical parameters as compared to ethanol control (Table 2).

Table 1: Effects of methanolic extract of *H. vulgare* seeds on physical and functional parameters

Groups	Thiopentone induced sleeping		Mean liver weight (g/100g)	Mean liver volume (ml/100g)
	Onset (sec)	Duration (min)		
Group-I	172.67±3.55	41.67±1.05	3.84±0.30	4.42±0.33
Group-II	118.33±4.59$^{\#\#}$	144.00±6.56$^{\#\#}$	5.44±0.14$^{\#\#}$	5.35±0.18$^{\#\#}$
Group-III	150.83±3.94*	89.50±3.49*	3.87±0.24*	4.08±0.35*
Group-IV	157.83±2.80 *	75.83±2.26*	3.78±0.07*	3.92±0.30*
Group-V	161.00±1.77*	58.33±2.33*	3.80±0.15*	3.58±0.24*

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; * P<0.05 is considered significant when compared with group II by Dunnett's multiple comparison test.

Table 2: Effects of methanolic extract of *H. vulgare* seeds on various serum biochemical parameters

Biochemical Parameters	Group-I	Group-II	Group-III	Group-V	Group-IV
SGOT (IU/L)	33.50 ±4.69	169.67 ±12.40$^{\#\#}$	116.33 ±7.73*	96.00±7.15*	78.00±7.66*
SGPT (IU/L)	28.17±2.17	125.33±6.80$^{\#\#}$	64.33±3.01*	60.00±3.25*	47.00±5.23*
ALP(KAU/dl)	7.18 ±0.71	99.67± 6.84$^{\#\#}$	34.54 ±3.04*	27.29 ±2.63*	20.49±1.95*
TBL (mg/dl)	0.36±0.04	1.53±0.14 $^{\#\#}$	0.69 ±0.08*	0.52 ±0.08*	0.42 ±0.06*
DBL (mg/dl)	0.14 ±0.02	0.84 ±0.04$^{\#\#}$	0.52±0.08*	0.40 ±0.05*	0.29±0.03*
TC(mg/dl)	89.50±4.71	170.10±15.28$^{\#\#}$	106.53±3.02*	91.26±7.81*	94.32±4.14*
TG(mg/dl)	65.59±8.78	217.56±3.41$^{\#\#}$	117.68±1.99*	101.55±11.00*	88.51±8.65*
TP (mg/ml)	7.69±0.77	2.99±0.64 $^{\#\#}$	5.99 ±0.51*	6.11 ±0.15 *	6.07±0.11*

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; * P<0.05 is considered significant when compared with group II by Dunnett's multiple comparison test.

Histological examination of the normal control showed a normal architecture of the liver with distinct hepatic cells, sinusoidal spaces and central vein (Fig 1A). In ethanol control, increased the scores of fatty degeneration and periportal fibrosis was observed (Fig 1B). Treatments with Test-1, Test-2 and positive control preserved the normal structure of liver (Fig 1C, 1D and 1E) by significantly reducing the scores of fatty degeneration and periportal fibrosis with evidence of significant regeneration (Table 3).

Table 3: Effects of methanolic extract of *H. vulgare* seeds on liver histological score in ethanol-induced liver damage

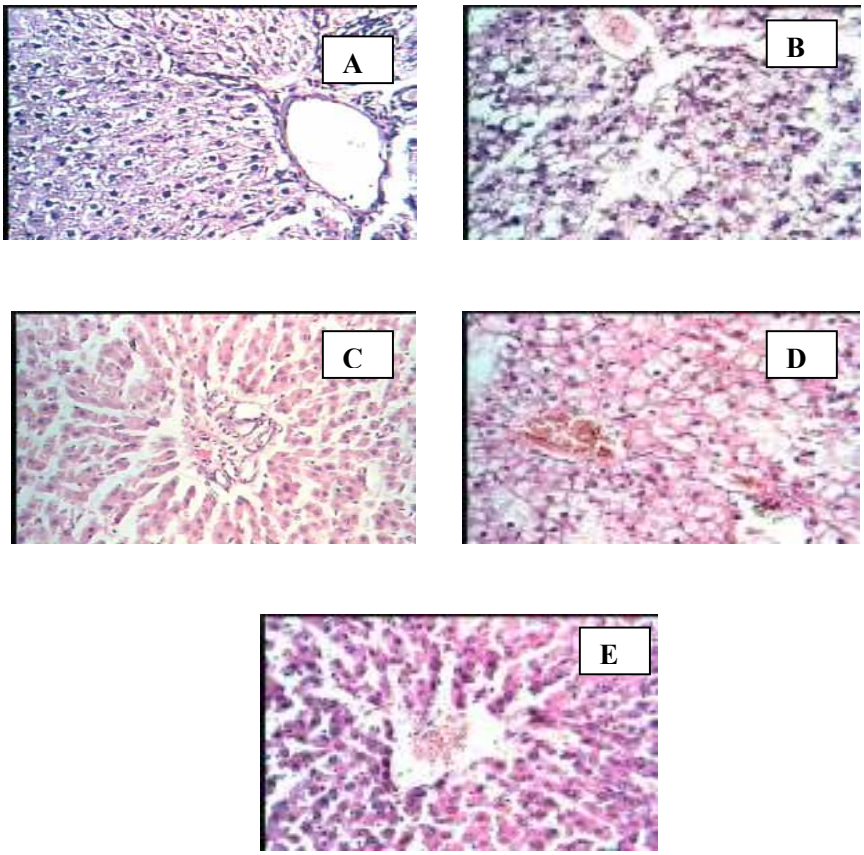
Histological parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
Fatty degeneration	0	2.83± 0.17$^{\#}$	1.5 ± 0.22*	0.67 ± 0.21**	0.5 ± 0.22**
Periportal fibrosis	0	2.67 ± 0.21$^{\#}$	1.33 ± 0.21*	0.67 ± 0.21**	0.5 ± 0.22**
Regeneration	0	0	1.33±0.21**	1.67 ± 0.21**	1.83 ± 0.17**

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<0.01 are considered significant when group II compared with group I; *P<0.01 and ** P<0.005 are considered significant when group II compared with group III, group IV and group V.

Figure 1: Photomicrograph of rat liver obtained from different treatment groups



A: Normal control, B: Ethanol control, C: Test-1, D: Test-2, E: Positive control (H and E, 100X)

Discussion

The liver can be injured by many chemicals and drugs. Ethanol was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. Ethanol produces dose-related deleterious effects in the liver [22]. Ethanol alters the metabolic activity of hepatocytes, thereby inducing hepatic damage. Barbiturates are a class of xenobiotics that are extensively metabolized in the liver. Deranged liver function leads to delay in the clearance of barbiturates, resulting in a longer duration of hypnotic effect [23]. In the present study, administration of thiopentone sodium to rats pretreated chronically with ethanol resulted in an increased duration of thiopentone sodium sleep time. Treatments with methanolic extract of *H. vulgare* seeds significantly decreased thiopentone-induced sleep time, an indirect evidence of their hepatoprotective activity.

In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes [24], with an impaired protein secretion by hepatocytes [25]. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume [26]. Treatments with methanolic extract of *H. vulgare* seeds significantly reduced the total wet-liver weight and volume, thus indicating their hepatoprotective activity.

During hepatic damage, cellular enzymes like SGOT, SGPT and ALP present in the liver cells leak into the serum, resulting in increased concentrations [27].

Ethanol administration for 18 days significantly increased all these serum enzymes whereas treatments with methanolic extract of *H. vulgare* seeds significantly reduced SGOT, SGPT, ALP and increased TP levels indicating their hepatoprotective activity.
Ethanol also induces hypercholesteremia and hypertriglyceridemia, which may be due to the activation of enzyme HMG Co-A reductase, the rate-limiting step in cholesterol biosynthesis. The increased serum triglyceride level in ethanol control may be due to the decreased activity of lipoprotein lipase, which is involved in the uptake of triglyceride rich lipoprotein by the extra hepatic tissues [28]. Treatments with methanolic extract of *H. vulgare* seeds significantly reduced the TC and TG levels, suggesting that the extracts prevented ethanol-induced hyperlipidemia probably due to their hepatoprotective activity.

Histological changes such as fatty degeneration and periportal fibrosis were observed in ethanol control. Treatments with methanolic extract of *H. vulgare* seeds prevented these histological changes, further indicating their hepatoprotective activity.

Ethanol, even after short-term consumption, induces CYP2E1 enzyme activity in doses that do not cause fatty changes. This enzyme accelerates alcohol metabolism with a resultant increase in acetaldehyde production [29]. Acetaldehyde is thought to have a number of adverse effects like decreased transport and secretion of proteins due to tubulin polymerization, enhanced vitamin metabolism and trace metals and drugs like paracetamol cause severe acute liver injury which is sometimes fatal [30-32]. Antioxidants exhibit hepatoprotective activity by blocking the conversion of ethanol to acetaldehyde [33].

Phytoconstituents like Phenolic compounds (flavonoids) [34], terpenoids [35], glycosides [36] and saponins [37] are well known for their antioxidant and hepatoprotective activities. Preliminary phytochemical screening of methanolic extract of *H. vulgare* seeds contain above phytoconstituents which may be attributed to the individual or combined effect of phytoconstituents present in it. Further, investigation is underway to determine the exact phytoconstituents that is responsible for the activity.

It can be concluded that methanolic extract of *H. vulgare* seeds possessed the hepatoprotective activity against ethanol-induced liver damage in rats, as evidenced by the functional, physical, biochemical and histological parameters.

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