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PHARMACOLOGICAL EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF HORDEUM VULGARE

[1]Mohammad Zubair Shareef, [2*]Gummadi Sridhar Babu and [3]P. S. Malathy
[1]Mother Teresa College of Pharmacy, NFC Nagar, Hyderabad.
[2*, 3] Sri Shivani College of Pharmacy, Mulugu Road, Warangal.

*Corresponding Author Email: sridhbabu@gmail.com

ABSTRACT

The present study was the main objective to assess the hepatoprotective activity of hydroalcoholic extract of root of Hordeum vulgare. LD50 studies were conducted in albino rats with hydroalcoholic extract of root of Hordeum vulgare according to OECD guideline No.423 and were found safe upto the dose level of 2gm/kg confirming its non-toxic nature. The Hepatoprotective activity was studied in carbon tetra chloride, paracetamol and ethylene glycol induced hepatotoxic animal model. The biochemical parameters like serum SGPT, SGOT, albumin, decreases and total protein increases with hydroalcoholic extract of root of Hordeum vulgare root confirmed the hepatoprotective effect of extract under this study. In liver injury models in rats restoration of hepatic cells with minute fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepato protection. Mainly based on the improvement in serum marker enzyme levels it was concluded hydroalcoholic extract of root of Hordeum vulgare possesses significant hepatoprotective activity in the doses used.

KEY WORDS

Ocimum tenuflorium, Hhepatoprotectivity, Silymarin

INTRODUCTION

Traditional medicine using herbal drugs exists in every part of the world. The major areas are Chinese, Indian and European traditions the philosophies of this traditional medicine have some resemblance to each other but differ widely from modern western medicine. The western medicine are not only new synthetic drugs, but also herbal drugs have to fulfill the international requirements for Quality, Safety and efficacy. [1] The Indian medicine system Ayurveda came into existence. The raw materials for Ayurvedic medicines were mostly obtained from plant sources in the form of crude drug such as dried herbal powders.[2] The Siddha, Unani and tibia are traditional health care system which has been flourishing for many centuries.[3]

We, the human being possesses a huge wealth of medicinal plants which have been explored and validated for their therapeutic properties. Still there are so many plants whose medicinal properties are not yet published and lots of research works are needed to be carried out on such medicinal plants. Herbal drugs play a vital role in the management of various liver disorder, most of them speed up the natural healing process of liver.[4] Numerous medicinal plants and their formulations are used in liver disorders in ethno medicinal practices as well as traditional system of medicine in India. According to WHO about 18,000 people die every year due to hepatic disease. Liver diseases remain one of the serious health problems is the absence of variable liver protective drugs. The common ailments of liver are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, hepatic failure and certain tumours like hepatoma.[5]

Various types of treatment modalities are available to treat liver diseases. In allopathic medical practices, herbs play role in the management of various liver disorders. Since however, we do not have satisfactory remedy for disorders of liver, the search for finding out effective hepatoprotective drugs continues. Many unknown and lesser known plants are used is folk and tribal medical practices in India. The medicinal values of these plants are not known to the scientific world. The present work deals with hepatoprotective activity of ethanolic extract of root of *Hordeum vulgare* (Gaerth) against carbon tetrachloride, ethyl alcohol and paracetamol induced liver damage in rats.[6]

Barley (Hordeum vulgare L.), a member of the grass family, is a major cereal grain grown in temperate climates globally. It was one of the first cultivated grains, particularly in Eurasia as early as 13,000 years ago. Barley has also been used as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. It is used in soups and stews, and in barley bread of various cultures. Barley grains are commonly made into malt in a traditional and ancient method of preparation.[7] Hordeum vulgare is an annual growing to 1 m (3ft 3in) by 0.2 m (0ft 8in).

It is hardy to zone (UK) 4 and is not frost tender. It is in flower from Jun to August. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Wind.Suitable for: light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. Suitable pH: acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It prefers moist soil. The plant can tolerate strong winds but not maritime exposure.[8]

The shoots are diuretic. The seed sprouts are demulcent, expectorant, galactofuge, lenitive and stomachic. They are sometimes abortifacient. They are used in the treatment of dyspepsia caused by cereals, infantile lacto-dyspepsia, regurgitation of milk and breast distension. They are best not given to a nursing mother since this can reduce milk flow. The seed is digestive, emollient, nutritive, febrifuge and stomachic. It is taken internally as a nutritious food or as barley water (an infusion of the germinated seed in water) and is of special use for babies and invalids. Its use is said to reduce excessive lactation. Barley is also used as a poultice for burns and wounds. The plant has a folk history of antitumour activity. The germinating seed has a hypoglycaemic effect preceded by a hyperglycaemic action. Modern research has shown that barley may be of aid in the treatment of hepatitis, whilst other trials have shown that it may help to control diabetes. Barley bran may have the effect of lowering blood cholesterol levels and preventing bowel cancer. Other uses for bronchitis and diarrhoea, and as a source of folic acid and vitamin B12 and B6. Weight loss. [9-11]

EXPERIMENTAL WORK

Animals

Male Wistar albino rats Weight about 150-200 cm. were used for the study. The animals were housed in groups of six and maintained under standard conditions (27±2ºC, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours, respectively) and fed with standard rat diet and purified drinking water and libitum for 1 week before and during the experiments.[12]

All experiments and protocols described in the present study were approved by the Institutional Animal Ethical Committee (IAEC) of and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals. Ministry of Social Justice and Empowerment, Government of India.

Plant Material

Collection of plant material

The plant *Hordeum vulgare* was collected in Tirumala forests, Tirupati, A.P, India in the month of January 2015.

Preparation of *Hordeum vulgare* root extract[87]

Soxhalation process:

The powdered plant materials were placed in the thimble present in the central compartment, with a siphoning device side arm which was connected to the lower compartment. The solvent was placed in central and lower compartments. Then the solvent was heated to boiling. The solvent vapor, which was generated by gently heating the reservoir gets condensed and was allowed to drip back into the porous sample cup. The liquid condensate that drips onto the sample performs the extraction which then passed through the container and back into the reservoir. The cycle was repeated continuously and can be sustained as long as needed. As it progresses, the species were concentrated in the reservoir. The powdered plant material was subjected to the extraction process by 50% alcohol and 50% of distilled water (500 ml/100g of dried powder) for 18 hrs. The extract solutions obtained were collected separately and concentrated using a rotary evaporator. The yield of the Hydroalcoholic extracts was found to be 2.5 % and 2.5 % (w/w) respectively. The dried extracts were stored in an air tight container and placed in a refrigerator. [13-15]

Pharmacological studies

Acute Toxicity studies (OECD)

Acute oral toxicity study was performed as per OECD-423 guidelines. Wistar albino rats (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for an overnight provided with only water, after which the extract was administered orally at the dose level of 2000 mg/kg body weight by gastric incubations and observed for 24 hours. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 3000 mg/kg of body weight.[16]

Animals were observed continuously for 2 hours under the following aspects.

Behavioral profile: Alertness, restlessness, irritability and fearfulness.

Neurological profile: Spontaneous activity, touch, response and gait.
Autonomic profile: Defecation and urination.
The mortality and morbidity were observed after 24 hours.

Hepatoprotective studies

Carbon tetrachloride (CCl_4) induced hepatotoxicity

Rats of either sex were divided into five groups of six animals in each group. (n = 6)
Group I: This group received 0.2% of Carboxy methyl cellulose solution (1ml/kg) once daily for nine days.
Group II: Received 0.2% of Carboxy methyl cellulose solution (1ml/kg) once daily for nine days and carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) on the 7th day.
Group III: Received standard drug silymarin (25 mg/kg, p.o.) for 9
days once daily and carbon tetrachloride (1 ml/kg in 50% v/v olive
oil, s.c.) on the 7th day.
Group IV: This group received *Hordeum vulgare* hydroalcoholic leaf extract (200mg/kg, BW/PO) for 9 days once daily carbon tetrachloride (1 ml/kg in50% v/v olive oil, s.c.) on the 7th day.
Group V: This group received *Hordeum vulgare* hydroalcoholic leaf extract (400mg/kg, BW/PO) for 9 days once daily carbon tetrachloride (1 ml/kg in50% v/v olive oil, s.c.) on the 7th day.
On the last day, serum marker enzyme parameters *i.e.,* Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT) and biochemical parameters Alkaline phosphatase (ALP)[91] and biochemical parameters i.e. Total bilirubin and Total protein[92] were analyzed according to the reported methods. [17]

Serum biochemical methods

1. Estimation of Aspartate amino Transferase (AST) or SGOT

Principle

SGOT catalyses the following reaction, i.e. the transfer of an amino group from L-Aspartate to α-ketoglutarate.
α- Ketoglutarate + L- Aspartate ↔ L- Glutamate + Oxalo-acetate
Oxaloacetate so formed is coupled with 2, 4-Dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydration which gives a brown color in alkaline medium and this can be measured calorimetrically.

Reagents

Reagent 1: Buffered Aspartate α-KG substrate pH: 7.4.
Reagent 2: DNPH color reagent.
Reagent 3: Sodium hydroxide, 4 N.
Reagent 4: Working Oxaloacetate standard, 2 mm.
Solution 1: Dilute 1 ml of Reagent 3 to 10 ml with purified water.

Procedure

Standard curve

As the reaction proceeds with time more amounts of products are formed and since the end products inhibit the enzyme, there is more of inhibition. This is the major problem with calorimetric methods for the estimation of this enzyme. On the other hand, in kinetic methods, since the enzyme activity is measured in the initial few minutes, the amount of products formed during the short time are negligible to cause any inhibition. Because of the above problem, it is necessary to standardize any calorimetric method against a standard kinetic method.
The standard graph is drawn with enzyme activity (IU/l) on the x-axis and O.D on y-axis and is not a linear one, which shows that O.D increases with increase in enzyme activity at a decreasing rate.

Table no. 1. Procedure of SGOT for Standard

Tube No.	1	2	3	4	5
Assigned enzyme activity (IU/l)	0	24	61	114	190
Reagent to be pipette	Volume in ml				
Reagent 1	0.5	0.45	0.4	0.35	0.3
Reagent 4	-	0.05	0.1	0.15	0.2
Purified water	0.1	0.1	0.1	0.1	0.1
Reagent 2	0.5	0.5	0.5	0.5	0.5
Mix well and allow to stand at room temperature (15 – 30 °C) for 20 minutes					
Solution 1	5.0	5.0	5.0	5.0	5.0

Mix well by inversion. Allow to stand at room temperature (15-30 °C) for 10 minutes and measure the O.D of all the five test tubes against purified water on a colorimeter with a green filter at 505 nm. [18]

Table no. 2. Procedure of SGOT for Test

Pipette into tube marked for test	Test (T)
Reagent 1	0.25 ml
Incubate at 37 °C for 5 minutes	

Serum	0.05 ml
Mix well and incubate at 37 °C for 60 minutes	
Reagent 2	0.25 ml
Mix well and allow to stand at room temperature for 20 minutes	
Solution 1	2.5 ml

Mix well by inversion. Allow to stand at room temperature (15-30 °C) for 10 minutes and measure the O.D of all the five test tubes against purified water on a colorimeter with a green filter at 505 nm.

Calculations

Mark the O.D of test on the Y-axis of the standard curve and extrapolate it to the corresponding enzyme activity on X-axis.

Estimation of Alanine amino Transferase (ALT) or SGPT

Procedure

Standard curve

As the reaction proceeds with time, more amounts of products are formed and since the end products inhibit the enzyme, there is more of inhibition. This is the major problem with calorimetric methods for the estimation of this enzyme. On the other hand, in kinetic methods, since the enzyme activity is measured in the initial few minutes, the amount of products formed during the short time are negligible to cause any inhibition. Because of the above problem, it is necessary to standardize any calorimetric method against a standard kinetic method.[19]

The standard graph is drawn with enzyme activity (IU/L) on the X-axis and O.D on Y-axis and is not a linear one, which shows that O.D increases with increase in enzyme activity at a decreasing rate.

Table no. 3 Procedure of SGPT for Standard

Tube No.	1	2	3	4	5
Assigned enzyme activity (IU/L)	0	28	57	97	150
Reagent to be pipette	Necessary Volume in ml				
Reagent 1	0.5	0.45	0.4	0.35	0.3
Reagent 4	-	0.05	0.1	0.15	0.2
Purified water	0.1	0.1	0.1	0.1	0.1
Reagent 2	0.5	0.5	0.5	0.5	0.5
Mix well and allow to stand at room temperature (15-30 °C) for 20 minutes					
Solution 1	5.0	5.0	5.0	5.0	5.0

Mix well by inversion. Allow to stand at room temperature (15-30 °C) for 10 minutes and measure the O.D of all the five test tubes against purified water on a colorimeter with a green filter at 505 nm.

Table no. 4 Procedure of SGPT for Test

Pipette into tube marked for test	Test (T)
Reagent 1	0.25 ml
Incubate at 37 °C for 5 minutes	
Serum	0.05 ml
Mix well and incubate at 37 °C for 30 minutes	
Reagent 2	0.25 ml
Mix well and allow to stand at room temperature for 20 minutes	
Solution 1	2.5 ml

Mix well by inversion. Allow to stand at room temperature (15-30 °C) for 10 minutes and measure the O.D of all the five test tubes against purified water on a colorimeter with a green filter at 505 nm.

Calculations

Mark the O.D of test on the y-axis of the standard curve and extrapolate it to the corresponding enzyme activity on x-axis.

3. Estimation of total protein (TP)

Procedure

Pipette into clean, dry test tubes labeled Blank (B), Standard (S), and Test (T)

Table no. 5. Procedure for Total protein

	B	S	T
Biuret Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	2.0 ml	2.0 ml	2.0 ml
Standard	-	0.05 ml	-
Serum	-	-	0.05 ml

Mix well and incubate at 37 °C for 10 minutes. Measure the absorbance of Standard (S), and Test (T) against Blank (B) with yellow-green filter on a colorimeter at 555 nm.

Calculations

$$\text{Total protein gm\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times \text{Std. conc}$$

Estimation of creatinine:

Turn on the spectrophotometer and let warm up for at least 15 minutes. Set the wavelength to 520 nm. Dilute urine specimens 1:10 with distilled deionized water. Precipitate the proteins present in the patient serum or urine specimens and in each control by adding 0.5 ml of the specimen to 4.0 ml tungstic acid in a test tube. Shake vigorously and centrifuge for 10 minutes. Label cuvettes 1 through 10. Add 1.0 ml of picric acid solution to cuvettes 1 through 10. Add 3.0 ml distilled deionized water to cuvette

Procedure

Add 3.0 ml of the 0.5 mg/dl creatinine standard to cuvette
Add 3.0 ml of the 1.0 mg/dl creatinine standard to cuvette
Add 3.0 ml of the 2.0 mg/dl creatinine standard to cuvette
Add 3.0 ml of the 4.0 mg/dl creatinine standard to cuvette
Add 3.0 ml of the 10.0 mg/dl creatinine standard to cuvette
Add 3.0 ml of the protein free centrifugate of control Level One to cuvette
Add 3.0 ml of the protein free centrifugate of control Level Two to cuvette
Add 3.0 ml of the patient's protein free serum or urine centrifugate to the remaining cuvettes.
Mix by inversion using a paraffin square to prevent spillage.
Add 0.5 ml of the NaOH solution to the first cuvette. Mix and set a timer for 15 minutes.
Add 0.5 ml of the NaOH to the remaining cuvettes at 30 second intervals.
After 15 minutes, place cuvette 1 in the spectrophotometer and set the Absorbance to read 0.000.
Read Absorbance at exactly 15 minutes after adding the NaOH and record the Absorbance for cuvettes 2-10.

Estimation of Albumin:

Procedure (Manual)

1. Label test tubes blank, standard, control, test, etc.
2. Pipette 1.0 ml of reagent into each tube.
3. Transfer 0.01 (10ul) of sample to respective tubes and mix.
4. Incubate all tubes at room temperature for one minute.
5. Zero spectrophotometer with the blank at 630 nm.
6. Read and record absorbance's of all tubes. *For spectrophotometers requiring greater than 1.0 ml to read, 3.0 ml of reagent and 20ul of serum should be used

Calculation

$$\text{Abs} = \frac{\text{Absorbance}}{\text{Abs. of Unknown}} \times \text{conc. of / Albumin (g / dl)}$$

Abs. of Standard Std

Statistical analysis

All the data were expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Dunnett's Multiple Comparison test using a computer-based fitness program (Prism, Graph pad.). Statistical significance was determined at $P < 0.001$.

RESULTS

The present study was investigated for Hepatoprotective activity of *Hordeum vulgare* against carbon tetra chloride, Paracetamol and ethanol induced hepatotoxicity in rats.

Estimation of Extraction:

The root of *Hordeum vulgare*. were get shade dried and coarsely powered. The course powdered was used for the biological screening and Phytochemical testing. The extraction process can be carried out by percolation process.

The extraction was done with different solvents such as aqueous, ethanol and hydroalcohol, instead of that which can get more yield it can be chosen for this study. The more yield got from the hydroalcohol.

Table. No.6 Parameters: Estimation of Extraction:

S.NO	Name of the plant extract	Practical yield	Theoretical yield	Percentage yield
1.	Aqueous extract	24gm	50gm	48%W/W
2.	Ethanolic Extract	20gm	50 gm	36%W/W
3.	Hydroalcoholic Extract	34gm	50gm	65%W/W

Toxicity study:

In the present study the Hydroalcoholic extract of *Hordeum vulgare* root was subjected for toxicity studies.

For the LD50 dose determination, Hydroalcoholic extract was administered upto dose 2gm/kg body weight and extract did not produce any mortality, thus $1/10^{th}$ (200mg), $1/20^{th}$ (400mg) of maximum dose tested were selected for the present study.

LD50 of extract of *Hordeum vulgare* were calculated and found to be 2000mg/kg.

Hepatoprotective activity

Chloroform induced toxicity

Effect of Hydroalcoholic extract of *Hordeum vulgare* on biochemical parameters in chloroform induced hepatotoxic rats.

Rats treated with chloroform developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and Albumin, Total protein and Creatinine when compared to normal control. Pre-treatment with Silymarin, hydro-alcoholic extract had showed good protection against chloroform induced toxicity to liver. Test group indicates a

significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which can be shown in the table no 7.

Table. No.7 Biochemical Parameters

S:NO	GROUPS	SGOT µ/l	SGPT µ/l	ALBUMIN g/dl	CREATININE g/dl	TOTAL PROTEIN mg/dl
1	Group I	89.43±3.121	76.14±1.25	5.143±.132	0.7620±0.0438	6.852±0.243
2	Group II	149.3±2.651***	106.4±2.36***	6.573±0.142**	1.543±0.0310***	3.056±1.932*
3	Group III	104.3±3.623***	73.6±3.01***	5.462±0.365**	0.843±0.0274***	6.561±0.316*
4	Group I V	136.9±2.43*	86.4±3.14***	6.052±0.174^{ns}	1.016±0.031***	5.318±0.241^{ns}
5	Group V	129.4±3.219***	79.3±3.62***	5.628±0.126*	0.897±0.030***	6.470±0.184*

All Values are expressed as mean ± SEM, One Way Analysis of Variance, followed by Dunnett's * P<0.05, ** P<0.01 & *** P<0.001 when compared with G II; G II is compared with G I;

SUMMARY AND CONCLUSION

The present study was the main objective to assess the hepatoprotective activity of hydroalcoholic extract of root of *Hordeum vulgare.* LD50 studies were conducted in albino rats with hydroalcoholic extract of root of *Hordeum vulgare* according to OECD guideline No.423 and were found safe upto the dose level of 2gm/kg confirming its non-toxic nature. The Hepatoprotective activity was studied in carbon tetra chloride induced hepatotoxic animal model. The biochemical parameters like serum SGPT, SGOT, albumin, decreases and total protein increases with hydroalcoholic extract of root of *Hordeum vulgare* root confirmed the hepatoprotective effect of extract under this study. In liver injury models in rats restoration of hepatic cells with minute fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepato protection. Mainly based on the improvement in serum marker enzyme levels it was concluded hydroalcoholic extract of root of *Hordeum vulgare* possesses significant hepatoprotective activity in the doses used.

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***Corresponding Author:**
Mohammad Zubair Shareef*
Email: mzubairshareef@gmail.com