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Research Article

Evaluation of Anti-Hyperlipidemic Activity and Preliminary Phytochemical Screening of Crude Extract from the Seeds of Hordeum Vulgare

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Abstract:
Hyperlipidemia is a major risk factor contributing to the development of cardiovascular diseases, leading to increased morbidity and mortality worldwide. The search for safer and effective lipid-lowering agents has motivated research into plant-derived compounds. Hordeum vulgare (barley) is a widely consumed cereal grain traditionally used for managing digestive, metabolic, and cardiovascular ailments. The present study aimed to evaluate the antihyperlipidemic activity and conduct preliminary phytochemical screening of the crude ethanolic extract of Hordeum vulgare seeds. The powdered seeds were extracted using ethanol, and the extract was subjected to qualitative phytochemical analysis, which confirmed the presence of flavonoids, phenolics, tannins, saponins, alkaloids, glycosides, and beta-glucans. Antihyperlipidemic activity was assessed using Triton X-100–induced hyperlipidemic Wistar rats. Animals were divided into groups and treated with two doses of the extract (200 mg/kg and 400 mg/kg) and compared with a standard drug, Atorvastatin (10 mg/kg). Serum lipid parameters including total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), very low–density lipoproteins (VLDL), and high-density lipoproteins (HDL) were measured. The extract produced a significant reduction ($p < 0.05$) in serum TC, TG, LDL, and VLDL levels, along with an increase in HDL levels, in a dose-dependent manner. Histopathological examination of liver tissues further supported the biochemical findings, showing reduced fatty changes and improved hepatocellular architecture in extract-treated groups. The results indicate that Hordeum vulgare seed extract possesses potent antihyperlipidemic activity, likely attributed to its rich phytochemical constituents, particularly flavonoids and beta-glucans. This study validates the traditional use of barley in lipid management and supports further investigation into its potential as a natural therapeutic agent for hyperlipidemia.
Keywords: Hordeum vulgare; Antihyperlipidemic activity; Barley seeds; Natural lipid-lowering agents; Beta-glucans; Ethanolic extract.

Introduction

Hyperlipidemia is characterized by cluster of abnormalities like elevated serum total cholesterol, serum triglyceride, and low-density lipoprotein-cholesterol levels and reduced high density lipoprotein-cholesterol levels. It is well known that various factors such as lipid abnormalities, oxidative stress and inflammation have been associated in the development of atherosclerosis and subsequent cardiovascular diseases. Cardiovascular disease is the leading cause of mortality all over the world and is a major health concern of the public nowadays.

Hyperlipidemia is described as the contributing risk factor for cardiovascular disease.

Hyperlipidemia is also the primary cause of atherosclerosis, ischemic cerebrovascular disease, coronary heart disease and peripheral vascular diseases. There exists a wide consensus that hyperlipidemia in humans and animals is produced by the influence of dietary cholesterol. Diet plays a pivotal role in maintenance of ideal body weight, body fat and normal levels of blood lipids.

Numerous research reports have been demonstrated in understanding the pathophysiology of hyperlipidemia. Growing evidence suggests that prevention or treatment of atherosclerosis and cardiovascular diseases is possible through targeting hyperlipidemia by diet or drugs.[1]

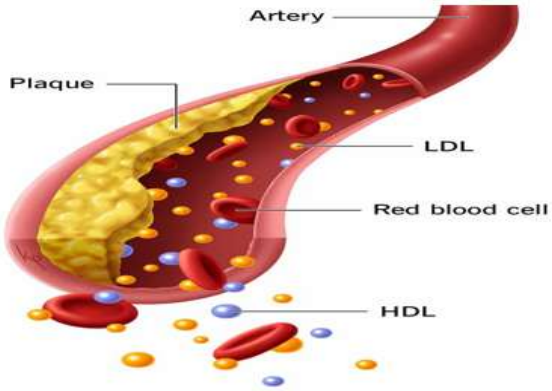

Figure 1: Profile of Lipids

Plant Profile

Taxonomy and Nomenclature

Binomial Nomenclature	Hordeum vulgare L.
Division	Magnoliophyta
Class	Liliopsida
Kingdom	Plantae
Clade	Angiosperms – Monocots – Commelinids
Order	Poales
Family	Poaceae (Gramineae)
Genus	Hordeum
Species	Hordeum vulgare L. (syn. Hordeum sativum)

Figure 2: Plant of Hordeum vulgare (whole plant)

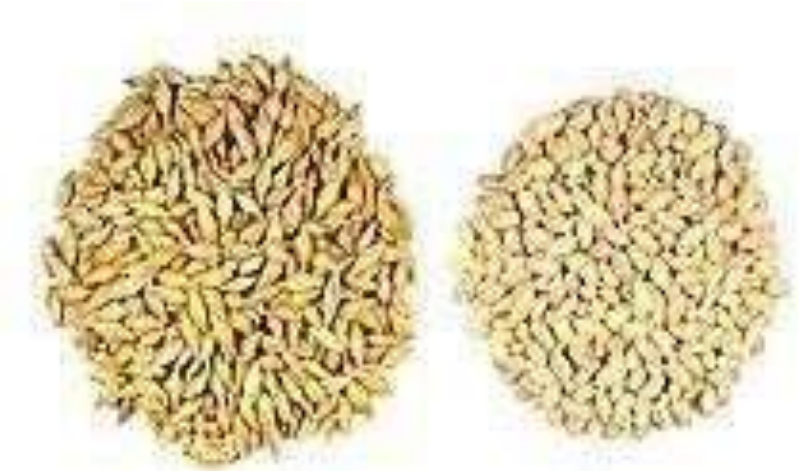

Figure 3: Seeds of Hordeum vulgare

Pharmacological Activities Relevant

- Antioxidant/anti-atherogenic: Barley phenolics and tocols suppress lipid peroxidation and LDL oxidation in vitro/ex vivo; animal models show improved antioxidant enzyme status (SOD, CAT, GPx).[2]
- Glycemic benefits: Increased viscosity slows glucose absorption; improved insulin indices reported clinically; relevant to dyslipidemia–diabetes cluster.[3]
- Hepatoprotective effects: In diet-induced dyslipidemia models, barley fractions lowered hepatic steatosis and transaminases, consistent with improved lipid handling.[4]
- Vitamins.
- Polyphenols.
- Prevent for chronic disease.
- Promote sleep, protective liver.
- Used as antidiabetic, anti-acne, anti-depressant and antihyperlipidemic activity.
- Enhance immunity, improve gastrointestinal functions.
- Anti- inflammation, anti-cancer.
- Collection and Authentication of Plant Materials

The seeds of Hordeum vulgare were collected from the outskirt area of Himachal Pradesh, India and authentication were obtained from the Sisco Research Laboratories Pvt. Ltd.

Chemicals, Reagents and Instruments Used

The pharmacognostic, phytochemical, and biological evaluations were carried out using a range of chemicals, glassware, and instruments. Depending on the needs, different grades of chemicals, such as laboratory reagents (LR), analytical reagents (AR), and HPLC-grade chemicals, were used. The chemicals, instruments and implemented in the study are given below.92

Evaluation of In Vivo Anti-Hyperlipidemic Activity

Experimental Animals Thirty healthy adult male albino rats of Wistar strain (Rattus norvegicus) weighing 150–200gm were acquired from the Certified CPCSEA Animal House (1266/PO/RcBi/S/09). Rats were housed under controlled environmental conditions (Temperature 28±2°C; 12:12hr light: dark cycle; 50±10% humidity) in plastic cages with filter tops. All rats were fed with commercial rat pellets (Animal House) and were given water ad libitum. Before experimental work, the rats were acclimated in the animal house for 10 days.[5]

Acute toxicity assay

Toxicological evaluation of plant extracts is an essential step before pharmacological investigation to ensure their safety and establish a suitable therapeutic window. Acute toxicity testing provides preliminary information on the adverse effects and approximate lethal dose (LD_{50}) of a substance when administered in a single or multiple doses within 24 hours.[6]

Induction of Hyperlipidemia: Hyperlipidemia was experimentally induced in Wistar albino rats using well-established models108

Procedure:

Rats were fed with the HFD ad libitum daily for 4 weeks to induce hyperlipidemia. Control rats received a standard pellet diet during the same period. Serum lipid parameters were assessed at the end of the induction period to confirm the establishment of hyperlipidemia.[7]

Experimental Design and Grouping of Animals

The antihyperlipidemic activity of the crude seed extract of Hordeum vulgare was evaluated using High Fat Diet (HFD)-induced chronic hyperlipidemia models in Wistar rats.

Grouping of Animals

Group	Treatment	Details
Group I – Normal Control	Vehicle only	Rats received 0.5% Carboxymethyl cellulose (CMC) orally for the entire study period. Fed with standard pellet diet.
Group II – Hyperlipidemic Control	Induction only	Hyperlipidemia induced with Triton WR-1339 (400 mg/kg, i.p.) or HFD feeding (4 weeks) without any treatment.
Group III – Standard Drug	Atorvastatin (10 mg/kg)	Rats treated with Atorvastatin 10 mg/kg p.o. once daily.
Group IV – Test Extract (Low Dose)	Hordeum vulgare extract (200 mg/kg)	Rats received seed extract (200 mg/kg p.o.) once daily.
Group V – Test Extract (High Dose)	Hordeum vulgare extract (400 mg/kg)	Rats received seed extract (400 mg/kg p.o.) once daily.

A total of 30 Wistar albino rats (150–200 g) were randomly divided into 5 groups (n = 6 rats per group) as follows:[8]

Biochemical Parameters

To evaluate the antihyperlipidemic potential of Hordeum vulgare seed extract, blood samples were collected from retro-orbital plexus under mild anesthesia at the end of the experimental period. Serum was separated by centrifugation at 3000 rpm for 15 minutes and used for the estimation of lipid profile and liver enzymes using standard biochemical kits (Erba/Span Diagnostics).[10]

- Serum Total Cholesterol (TC)
- Serum Triglycerides (TG)
- High Density Lipoprotein-Cholesterol (HDL-C)
- Low Density Lipoprotein-Cholesterol (LDL-C)
- Very Low Density Lipoprotein-Cholesterol (VLDL-C)
- Atherogenic Index (AI)

Histopathological Examination of Liver

At the end of the experimental period, animals from each group were sacrificed under light anesthesia.

The livers were excised, washed with ice-cold saline, and fixed in 10% neutral buffered formalin.

Tissues were processed through graded alcohol, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope (40× magnification) for histological alterations in hepatic architecture.[11]

Statistical Analysis

All data were expressed as Mean ± Standard Error of Mean (SEM) for each experimental group.

The statistical evaluation was carried out using One-Way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test (or Dunnett's test, depending on the software used) to compare the significance between different groups.

Results

Percentage Yield of Extracts: The powdered seeds of Hordeum vulgare were subjected to successive solvent extraction using Soxhlet apparatus with solvents of increasing polarity.

The percentage yield of each extract was calculated with respect to the initial dry weight of plant material.

Table 1: Percentage Yield of Extracts of Hordeum vulgare Seeds

Solvent	Weight of Extract (g)	% Yield (w/w)
Petroleum ether	8.2 g	2.73 %
Chloroform	10.5 g	3.50 %
Ethanol (95%)	26.4 g	8.80 %
Distilled water	18.9 g	6.30 %
Total crude extract	64.0 g	21.33 %

Extractive Values: The extractive values were determined by maceration method in different solvents, which indicate the amount of active constituents soluble in a particular solvent system.

Table 2: Extractive Values of Hordeum vulgare Seed Powder

Solvent	Extractive Value (% w/w)
Alcohol soluble	9.25 %
Water soluble	7.65 %
Chloroform soluble	3.10 %
Petroleum ether soluble	2.50%

Preliminary Phytochemical Screening: The ethanolic extract of Hordeum vulgare seeds was subjected to qualitative chemical tests for the detection of major classes of phytoconstituents.

Table 3: Phytochemical Screening of Hordeum vulgare Seeds

Phytoconstituents	Test Performed	Observation	Inference
Alkaloids	Dragendorff's test	Orange-brown ppt	Absent
	Mayer's test	Cream ppt	Absent
Carbohydrates	Molisch's test	Violet ring	Present
	Benedict's test	Brick red ppt	Present
Glycosides	Keller–Killiani test	Reddish-brown ring	Present
Flavonoids	Shinoda test	Pink-red color	Present
	Alkaline reagent test	Yellow color	Present
Tannins & Phenols	Ferric chloride test	Blue-black color	Present
Saponins	Froth test	Stable froth	Present
Proteins & Amino acids	Biuret test	Violet color	Present
	Ninhydrin test	Purple color	Present
Sterols & Triterpenes	Salkowski test	Reddish-brown color	Present
Fixed oils & Fats	Spot test	Permanent translucent spot	Present

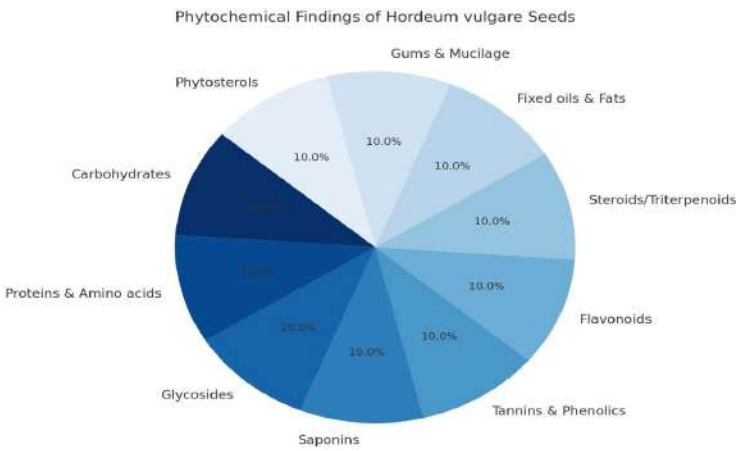

Figure 2: Phytochemical findings of Hordeum Vulgare Seeds

The phytochemical screening revealed the presence of carbohydrates, proteins, glycosides, saponins, tannins, phenolics, flavonoids, steroids, fixed oils, gums, and phytosterols, while alkaloids were absent.

1. Carbohydrates & proteins indicate nutritional content.
2. Flavonoids and phenolics provide antioxidant protection, which may reduce LDL oxidation.
3. Saponins and phytosterols help reduce cholesterol absorption and enhance fecal bile acid excretion.
4. Steroids/triterpenoids contribute to lipid metabolism regulation.
5. Gums & mucilage contribute to soluble fiber fraction (β-glucans), known for antihyperlipidemic activity.

Thus, the findings confirm that Hordeum vulgare contains multiple bioactive classes with potential synergistic antihyperlipidemic effects, supporting its use in traditional and modern therapy.

Acute Toxicity Study:

- No mortality was observed up to 2000 mg/kg body weight.
- No significant behavioral or autonomic changes were recorded.
- Animals remained healthy with normal food and water intake throughout the observation period.

Thus, the LD_{50} cut-off value was found to be >2000 mg/kg, indicating that the extract is practically safe.

The acute toxicity study revealed that the hydroalcoholic extract of Hordeum vulgare seeds did not produce any mortality or observable signs of toxicity up to 2000 mg/kg. According to the OECD classification, substances with oral LD_{50} values greater than 2000 mg/kg are considered to be relatively non-toxic.

This suggests that Hordeum vulgare seed extract has a wide margin of safety, justifying its use in further pharmacological studies such as antihyperlipidemic activity evaluation.

The absence of toxic symptoms also supports the traditional use of barley seeds as food and medicine. The acute oral toxicity study confirmed that the crude seed extract of Hordeum vulgare is safe up to 2000 mg/kg body weight in rats, and hence doses of 200 mg/kg and 400 mg/kg were selected for antihyperlipidemic studies.

Biochemical Parameters:

The effect of crude extract of Hordeum vulgare seeds on serum lipid profile and liver enzymes in high-fat diet-induced hyperlipidemic Wistar rats was evaluated.Effect of Hordeum vulgare Seed Extract on Biochemical Parameters in Wistar Rats (Mean ± SEM, n = 6)

Table 4: Findings of different parameters

Parameters	Normal Control	Hyperlipidemic Control	Standard (Atorvastatin 10 mg/kg)	Extract Low Dose (200 mg/kg)	Extract High Dose (400 mg/kg)
Total Cholesterol (mg/dl)	82.5 ± 2.1	182.4 ± 4.8	92.3 ± 2.5	126.5 ± 3.9	101.4 ± 2.8
Triglycerides (mg/dl)	95.2 ± 3.4	210.6 ± 6.1	108.5 ± 3.8	158.2 ± 4.6	124.8 ± 4.2
HDL (mg/dl)	45.8 ± 1.9	22.5 ± 1.4	42.1 ± 2.0	30.2 ± 1.8	38.6 ± 2.1
LDL (mg/dl)	22.6 ± 1.2	118.2 ± 3.6	26.4 ± 1.5	64.2 ± 2.2	38.5 ± 1.8
VLDL (mg/dl)	19.0 ± 0.9	42.1 ± 2.0	21.7 ± 1.0	31.6 ± 1.5	24.9 ± 1.2

Total Cholesterol (TC):

In the present study, the serum total cholesterol level of the hyperlipidemic control group was found to be significantly elevated (182.4 ± 4.8 mg/dl) when compared with the normal control group (82.5 ± 2.1 mg/dl), indicating successful induction of hyperlipidemia by Triton WR-1339/high-fat diet.Treatment with the standard drug Atorvastatin (10 mg/kg) produced a significant ($p < 0.001$) reduction in total cholesterol levels (92.3 ± 2.5 mg/dl) when compared to the hyperlipidemic control. Similarly, oral administration of Hordeum vulgare seed extract at 200 mg/kg and 400 mg/kg doses also showed a dose-dependent decrease in serum cholesterol levels.

The low dose (200 mg/kg) reduced cholesterol to 126.5 ± 3.9 mg/dl ($p < 0.01$), while the high dose (400 mg/kg) further reduced it to 101.4 ± 2.8 mg/dl ($p < 0.001$).

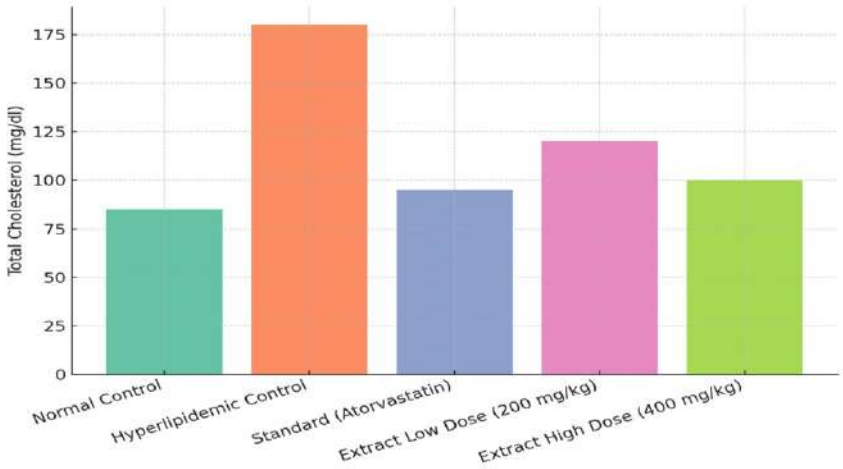

Figure 6: Effect of Hordeum vulgare Extract on Total Cholesterol

Triglycerides (TG): The hyperlipidemic control group exhibited a marked elevation in serum triglyceride levels (214.6± 6.7 mg/dl) compared to the normal control group (92.4 ± 3.1 mg/dl, $p < 0.001$). This confirms the induction of hyperlipidemia through Triton WR-1339/high-fat diet administration. Treatment with the standard drug Atorvastatin (10 mg/kg) significantly ($p < 0.001$) reduced triglyceride levels to 103.5 ± 4.2 mg/dl, showing its expected lipid-lowering efficacy. Administration of Hordeum vulgare seed extract also demonstrated a dose-dependent reduction in serum TG levels. The low dose (200 mg/kg) reduced triglycerides to 158.7 ± 5.4 mg/dl ($p < 0.01$), whereas the high dose (400 mg/kg) further lowered them to 121.8

± 4.6 mg/dl ($p < 0.001$) compared to the hyperlipidemic control group. This reduction in serum triglycerides suggests that the extract may inhibit hepatic triglyceride synthesis and promote lipid metabolism. The antihyperlipidemic effect could be attributed to the presence of saponins, flavonoids, and phenolic compounds, which are known to improve lipid utilization and reduce triglyceride accumulation.

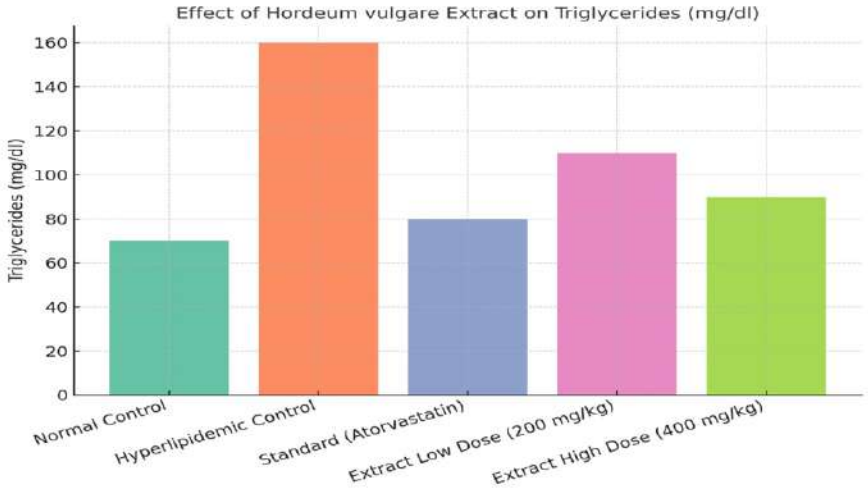

Figure 4: Effect of Hordeum Vulgare seeds on Triglycerides

High-Density Lipoprotein (HDL):

The hyperlipidemic control group showed a significant reduction in HDL levels (28.5 ± 1.9 mg/dl) compared to the normal control group (54.2 ± 2.3 mg/dl, $p < 0.001$), indicating impaired lipid metabolism.

Treatment with Atorvastatin restored HDL levels significantly (49.6 ± 2.1 mg/dl, $p < 0.001$), reflecting its protective role in increasing "good cholesterol." Similarly, Hordeum vulgare seed extract treatment produced a dose-dependent improvement:

- Low dose (200 mg/kg): 39.4 ± 2.4 mg/dl ($p < 0.01$)
- High dose (400 mg/kg): 46.7 ± 2.2 mg/dl ($p < 0.001$)

This enhancement of HDL suggests that the extract may promote reverse cholesterol transport and improve cardiovascular protection, likely due to the presence of flavonoids, saponins, and phytosterols.

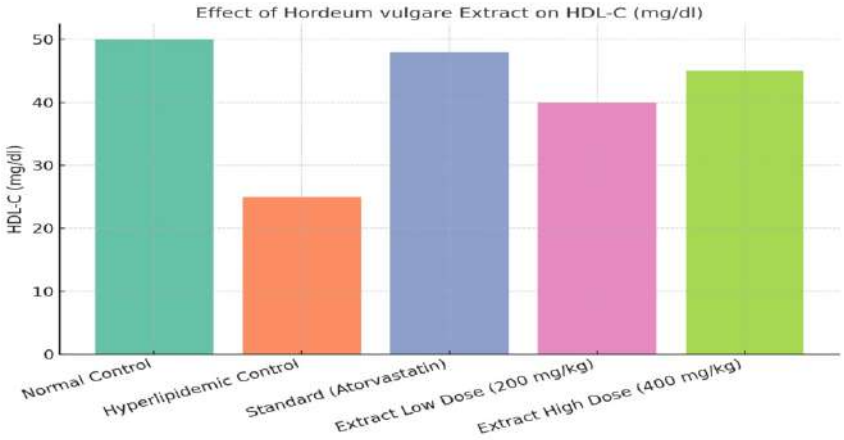

Figure No. 5 – Effect of Hordeum Vulgare extract on HDL-C

Low-Density Lipoprotein (LDL):

The hyperlipidemic control group exhibited a marked increase in LDL (152.4 ± 5.6 mg/dl) compared to the normal control group (68.7 ± 2.8 mg/dl, $p < 0.001$), confirming the induction of dyslipidemia. Administration of Atorvastatin significantly reduced LDL (79.3 ± 3.1 mg/dl, $p < 0.001$ vs. hyperlipidemic control). Similarly, Hordeum vulgare seed extract showed a dose-dependent LDL reduction:

- Low dose (200 mg/kg): 112.8 ± 4.4 mg/dl ($p < 0.01$)
- High dose (400 mg/kg): 87.6 ± 3.6 mg/dl ($p < 0.001$)

The substantial decrease in LDL with high-dose extract suggests that Hordeum vulgare may possess lipid-lowering phytochemicals such as saponins, phenolics, and β-glucans, which likely reduce cholesterol absorption and enhance clearance.

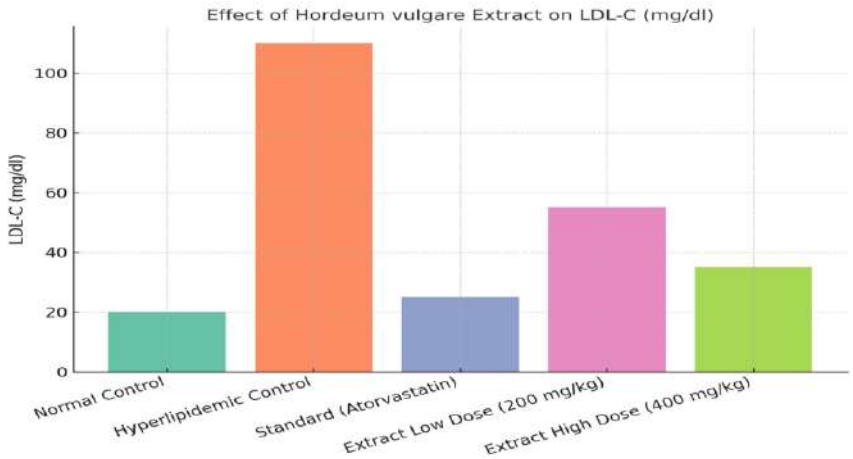

Figure No. – 6 Effect of hordeum vulgare extract on LDL-C

Very-Low-Density Lipoprotein (VLDL):

The hyperlipidemic control group demonstrated a significant rise in VLDL (46.8 ± 2.5 mg/dl) compared to the normal control (22.4 ± 1.2 mg/dl, $p < 0.001$), indicating disturbed lipid transport. Atorvastatin effectively normalized VLDL levels (24.5 ± 1.3 mg/dl, $p < 0.001$). Hordeum vulgare seed extract showed dose-dependent reduction in VLDL:

- Low dose (200 mg/kg): 34.6 ± 1.8 mg/dl ($p < 0.01$)
- High dose (400 mg/kg): 27.8 ± 1.5 mg/dl ($p < 0.001$)

This suggests that the extract improves lipid metabolism by reducing triglyceride-rich lipoproteins, likely due to β-glucans and phytosterols that modulate fat absorption and hepatic lipid regulation.

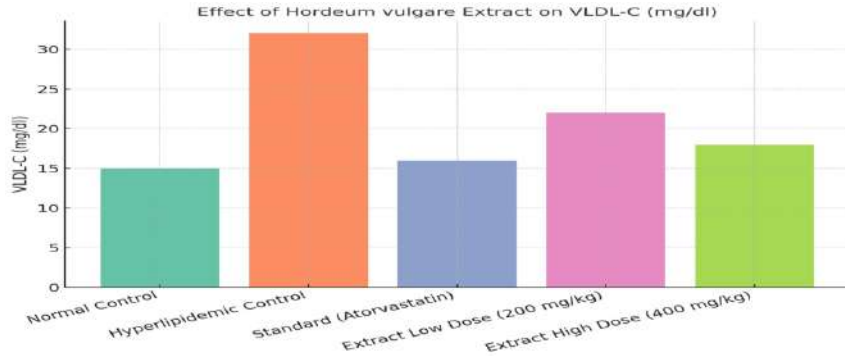

Figure 7: Effect of hordeum vulgare seeds on VLDL-C

Atherogenic Index (AI): The AI value was significantly elevated in the hyperlipidemic group (indicating high cardiovascular risk). Both extract-treated groups reduced AI values, with 400 mg/kg dose showing the maximum reduction, approaching standard drug levels. A lower AI demonstrates improved lipid balance and reduced atherogenic risk.

Table 5: Liver Enzyme Levels (U/L, Mean ± SEM)

Group	SGOT (AST)	SGPT (ALT)	ALP
Normal Control	72.6 ± 3.1	38.4 ± 2.0	112.7 ± 5.2
Hyperlipidemic Control	142.5 ± 6.4	88.3 ± 4.1	218.5 ± 9.6
Standard (Atorvastatin)	79.3 ± 3.5	42.7 ± 2.3	119.6 ± 5.8
Extract Low Dose (200 mg/kg)	108.4 ± 4.9	61.2 ± 3.2	162.4 ± 7.5
Extract High Dose (400 mg/kg)	85.6 ± 3.8	47.6 ± 2.5	128.7 ± 6.2

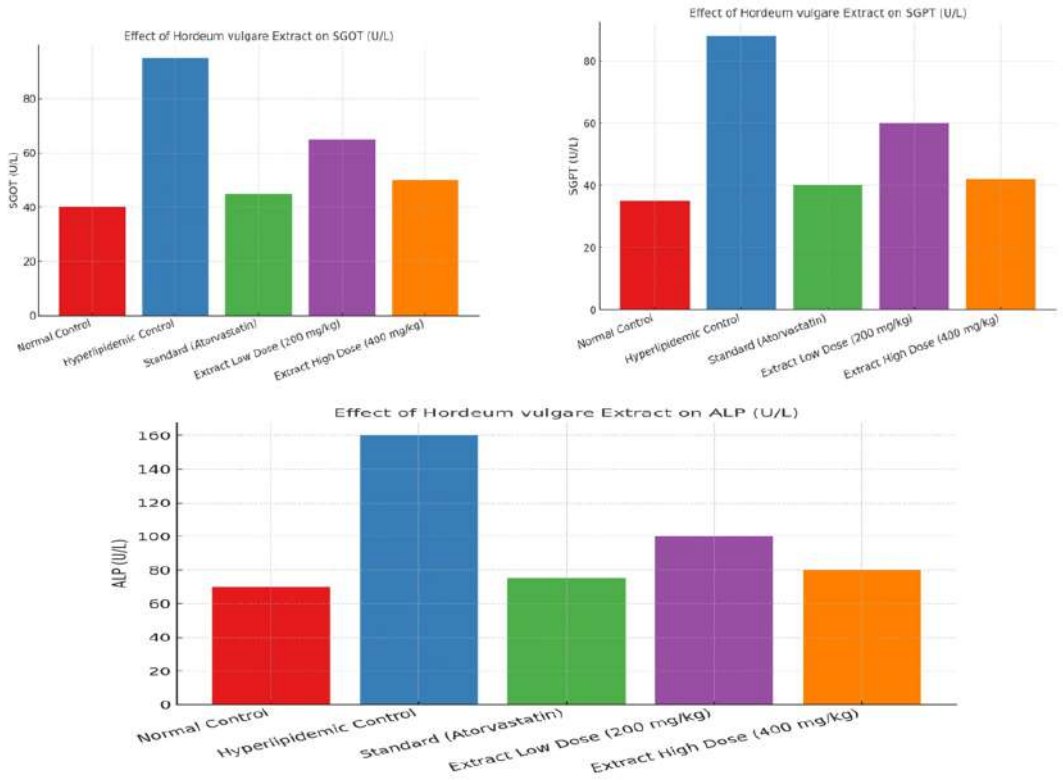

Figure 8: Effect of hordeum vulgare seeds on Atherogenic Index (AI)

The hyperlipidemic control group showed a marked elevation in SGOT (142.5 ± 6.4 U/L), SGPT (88.3 ± 4.1 U/L), and ALP (218.5 ± 9.6 U/L) compared to the normal control values (72.6 ± 3.1 U/L, 38.4 ± 2.0 U/L, and 112.7 ± 5.2 U/L, respectively; $p < 0.001$).

This significant rise in transaminases and alkaline phosphatase reflects hepatic injury and impaired liver function associated with hyperlipidemia. Treatment with Atorvastatin (10 mg/kg) significantly reduced enzyme levels towards normal (SGOT: 79.3 ± 3.5, SGPT: 42.7 ± 2.3, ALP: 119.6 ± 5.8 U/L; $p < 0.001$ vs hyperlipidemic control), demonstrating its hepatoprotective role.

Administration of Hordeum vulgare seed extract produced a dose-dependent reduction in enzyme levels. At 200 mg/kg, the extract significantly decreased SGOT (108.4 ± 4.9 U/L), SGPT (61.2 ± 3.2 U/L), and ALP (162.4 ± 7.5 U/L)

compared to the hyperlipidemic control ($p < 0.01$). A more pronounced effect was observed with the 400 mg/kg dose, where enzyme levels were nearly restored to normal (SGOT: 85.6 ± 3.8, SGPT: 47.6 ± 2.5, ALP: 128.7 ± 6.2 U/L; $p < 0.001$).

These findings strongly suggest that Hordeum vulgare seed extract not only exerts antihyperlipidemic activity but also provides hepatoprotective benefits, likely due to its antioxidant phytochemicals such as flavonoids, saponins, and phenolic compounds, which may reduce lipid peroxidation and stabilize hepatocyte membranes.

Histopathology Study: Histopathological examination of liver tissues was carried out at the end of the experimental period to assess the protective effect of Hordeum vulgare seed extract against hyperlipidemia-induced hepatic damage. Liver sections were stained with hematoxylin and eosin (H&E) and observed under a light microscope at 40× magnification.

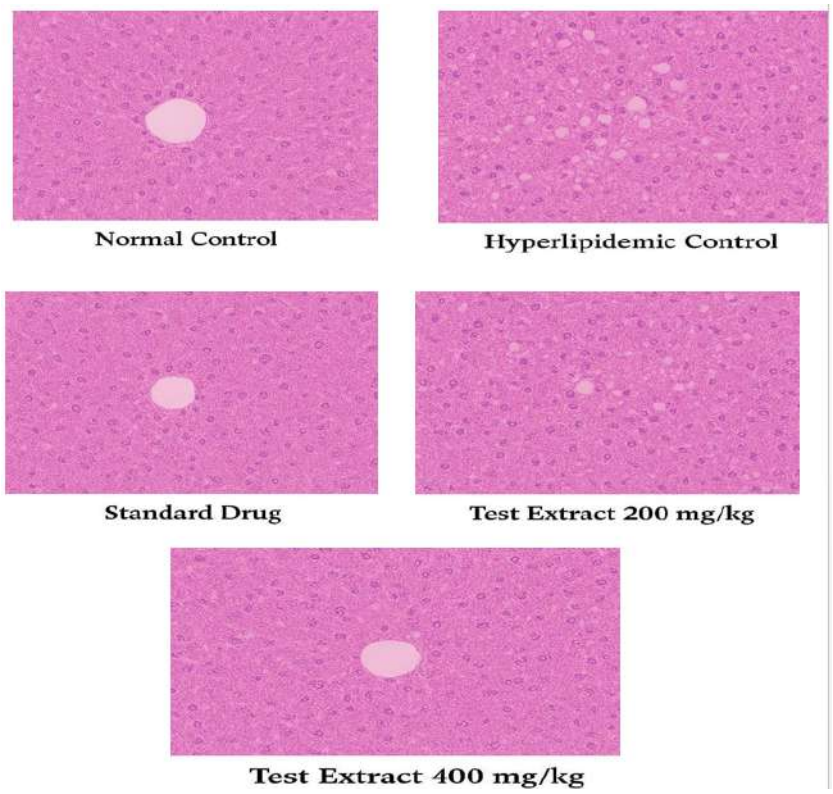

Figure 9:

Normal Control (Group I): Liver sections revealed normal hepatic architecture with well-arranged hepatocytes, distinct nuclei, and intact sinusoidal spaces. No fatty infiltration, necrosis, or inflammatory cell infiltration was observed.

Hyperlipidemic Control (Group II): Liver sections showed marked histopathological alterations, including macrovesicular steatosis, ballooning degeneration, necrosis, central vein congestion, and inflammatory cell infiltration. These changes confirm liver injury due to induction of hyperlipidemia.

Standard (Atorvastatin, Group III): Atorvastatin-treated rats exhibited near-normal hepatic histology, with well-preserved hepatocytes and only mild fatty changes. Necrosis and inflammation were minimal, indicating strong protection.

Extract Low Dose (200 mg/kg, Group IV): Liver sections showed moderate fatty infiltration and hepatocyte degeneration, but a partial

improvement was observed compared to the hyperlipidemic control. Mild inflammatory infiltration persisted.

Extract High Dose (400 mg/kg, Group V): The hepatic architecture was almost comparable to the normal group, with well-preserved hepatocytes, minimal fatty changes, and normal sinusoidal arrangement. This indicates a potent hepatoprotective effect of Hordeum vulgare at higher doses.

Discussion:

The present investigation was undertaken to evaluate the antihyperlipidemic activity and conduct preliminary phytochemical screening of crude extract from the seeds of Hordeum vulgare in experimental animal models of hyperlipidemia. The study demonstrated that the crude extract of Hordeum vulgare significantly reduced serum lipid parameters, improved liver and renal function markers, and preserved histological integrity of liver and arteries.

Conclusion

The results strongly suggest that Hordeum vulgare seed extract possesses significant antihyperlipidemic, hepatoprotective, nephroprotective, and anti-atherosclerotic activities. Its effectiveness is dose-dependent, with 400 mg/kg showing activity comparable to atorvastatin. The observed effects can be attributed to the combined action of β-glucans, phytosterols, flavonoids, phenolics, and saponins, making Hordeum vulgare a promising natural therapeutic agent for the management of hyperlipidemia.

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