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Parmotrema Perlatum: A Lichen& Indian Spice –Evaluation Of Phytoconstituents & Hepatoprotective Potential Of Its Aqueous Extract

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ABSTRACT

Background: Common colloquial names for the lichen *Parmotrema perlatum*, which belongs to the *Parmeliaceae* family, include Kalpasi, Raathi puvvu, Kallu hoovu, and Chadila. It usually grows on rocks in temperate regions and flourishes in humid oceanic and suboceanic habitats. Bioactive components of *Parmotrema perlatum* include phenolic compounds, alkaloids, flavonoids, steroids, and derivatives of usnic acid. It has long been used as a spice in Indian cooking and in traditional medicine to treat ailments like dermatitis, asthma, respiratory, and inflammatory problems. These substances raise the possibility of pharmacological actions, which calls for more research.

Methods: To determine the active ingredients in an aqueous extract of *Parmotrema perlatum*, chemical testing, phytochemical screening, and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were performed. Furthermore, ethanol was utilized to cause liver damage in a Wistar albino rat model in order to assess in vivo hepatoprotective efficacy. The protective effect of the extract was evaluated by looking at the histological alterations in liver tissue. The typical reference medication for comparison was silymarin.

Results: Alkaloids, flavonoids, steroids, and cardiac glycosides were detected by phytochemical examination. Compounds with a variety of pharmacological properties, including 5,7-dihydroxy-4-methyl coumarin, Platambin 1,6-dione, Sigmasten 3,5-diene, 1,2-longidione, and Gamolenic acid, were discovered by GC-MS analysis. In ethanol-induced hepatotoxic Wistar rats, the aqueous extract of Parmotrema perlatum showed notable hepatoprotective action at a dose of 200 mg/kg.

Conclusion: *Parmotrema perlatum* aqueous extract exhibits significant hepatoprotective potential, most likely as a result of the presence of many bioactive phytochemicals that were discovered by GC-MS analysis. These results underline the lichen's potential for additional pharmacological research while confirming its traditional use in the treatment of liver-related conditions**.**

Keywords: *Parmotrema perlatum, GC-MS, Coumarins, Flavonoids, Hepatoprotective activity.*

1. INTRODUCTION

Numerous physiological problems, including viral infections, metabolic abnormalities, and excessive alcohol consumption can result to liver damage.[1] Liver damage can result from usage, obesity, poor eating habits, unhealthy lifestyle choices, and certain medications. Jaundice, ascites, and other issues could result from it manifesting as fatty liver, cirrhosis, fibrosis, or even liver failure. Some liver damage can be repaired with treatment, particularly while it's still early.

A class of naturally occurring polyphenolic chemicals that are extensively found in the kingdom of plants are called flavonoids. They are widely present in fruits, vegetables, teas, and medicinal herbs and have attracted a lot of interest due to their potential as a treatment, especially for protecting important organs like the liver. As the main organ responsible for detoxification, the liver is frequently exposed to a variety of toxins, medications, and metabolic waste products that can cause inflammation and oxidative stress. Flavonoids are essential for hepatoprotection, which helps maintain the structure and function of the liver in such harmful circumstances.

Antioxidant activity is one of the main ways flavonoids provide their hepatoprotective benefits. Reactive oxygen species (ROS) are produced by the liver during metabolism, particularly under diseased circumstances. By scavenging free radicals and lowering lipid peroxidation, which otherwise harms cellular membranes, flavonoids counteract these ROS. Furthermore, flavonoids strengthen the liver's inherent defences against oxidative damage by increasing the activity of endogenous antioxidant enzymes such glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD).

Flavonoids have strong anti-inflammatory effects in addition to their antioxidant qualities, which help to preserve the liver. Inflammatory reactions can worsen liver damage in diseases such fatty liver disease or hepatitis. Flavonoids block important inflammatory pathways including nuclear factor kappa B (NF-κB) and prevent the synthesis of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β. This lessens the inflammation, necrosis, and development of cirrhosis or fibrosis in the hepatocytes.

Flavonoids also help to stabilize the liver cells' membranes. Enzymes like alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) seep into the bloodstream as a result of cell membrane breakdown after hepatic damage. By preserving the integrity of hepatocyte membranes, flavonoids lessen enzyme leakage and act as a sign of better liver health.

Furthermore, the cytochrome P450 family, which is essential for drug metabolism and the bioactivation of several hepatotoxins, is one of the liver enzyme systems that flavonoids can alter. Flavonoids aid in lowering the production of toxic intermediates that can impair liver tissue by controlling these enzymes. Because of this, they are especially helpful in shielding the liver from harm brought on by drugs like alcohol, paracetamol, and environmental pollutants.

Flavonoids have antiviral and antifibrotic properties in some liver diseases, including viral hepatitis. One flavonoid that has been demonstrated to be effective in preventing viral replication and lowering liver inflammation is silymarin, which is derived from milk thistle. Furthermore, flavonoids prevent or lessen liver scarring by inhibiting the activation of hepatic stellate cells, which are in charge of collagen synthesis and fibrosis.

Finally, flavonoids help injured liver cells heal and regenerate. They trigger a number of cellular signaling pathways that are important in tissue repair and cytoprotection, including the Nrf2/ARE pathway. After injury, this regenerative action is essential for regaining liver function.

In both experimental and clinical settings, a number of flavonoids have shown significant hepatoprotective effects, including apigenin (from parsley and chamomile), luteolin (from green peppers and celery), silymarin (from milk thistle), quercetin (found in apples and onions), and kaempferol (from broccoli and tea). They are extremely useful in the treatment and prevention of a variety of liver illnesses due to their multifactorial mode of action, which includes antiviral, anti-inflammatory, antioxidant, and regenerative qualities.

Modern synthetic medications frequently lack effectiveness and can have negative side effects if used for an extended period of time. Numerous traditional medicinal plants have demonstrated encouraging liver-protective properties.[2] However, only a small percentage have been standardized or proven by science. Thus, finding and researching novel plant sources may result in the development of hepatoprotective drugs that are less harmful, more efficient, and more reasonably priced. Furthermore, by using bioactive phytoconstituents, this investigation promotes the preservation of ethnomedicinal knowledge and presents chances for the creation of innovative drugs.

Indian spices' abundance of bioactive substances with anti-inflammatory, detoxifying, and antioxidant qualities makes them a considerable contributor to hepatoprotective function. Ayurvedic medicine has long utilized spices such as clove (eugenol), ginger (gingerol), black pepper (piperine), turmeric (curcumin), and cumin (cuminaldehyde) to support liver health. For instance, curcumin lowers inflammation and oxidative stress in liver cells, and piperine increases the bioavailability of other beneficial substances. These spices aid in lowering liver enzyme levels, inhibiting lipid peroxidation, regulating detoxifying enzymes, and guarding against infections, alcohol, and toxin-induced liver damage. They may help avoid chronic liver disorders and support liver function when consumed regularly.

Parmotrema perlatum is a lichen that can be eaten and used as seasonings in Indian cuisine because to its health advantages. [3] Such as anti-inflammatory, wound-healing, and carminative qualities, among others. [4-5]

Hence the preliminary phytochemical tests given the positive results for flavonoids and a few other significant hepatoprotective secondary metabolites of *Parmotrema perlatum[*6] ,the goal of this study is to record phytoconstituents using GC-MS analysis and hepatoprotective activity of aqueous extract of *Parmotrema perlatum* [7-8]

2. MATERIALS AND METHODS

2.1 Collection of material: Black stone flower samples were purchased in local market of Trivendrum (City), Kerala (state), India then herbariums were authenticated by Dr. K. Madhava Setty, Associate professor, Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra pradesh, India as their Voucher specimen Numbers: 0776 for *Parmotrema perlatum* respectively at S.V. university Herbarium. All the chemicals used for extraction and estimations were purchased from Sigma Aldrich, Bengaluru.

2.2 Preparation of aqueous extract by cold maceration: Crude drug *Parmotrema perlatum* was collected and made into fine powder. Then the powdered drug is taken in 1:20 ratio that is 50 mg of powdered crude drug in 1000 ml of distilled water and it is left for overnight and then it is filtered using muslin cloth and filtrate is collected.Then solvent is removed through rotaevaporation.This extract is used for further study.

2.3 Phytochemical investigation: Phytochemical tests were performed to identify secondary metabolites like alkaloids, glycosides, saponins, tannins flavonoids etc using respective reagents.The results obtained were tabulated in the manuscript.

2.4 GC-MS study: The Mass Selective Detector (MSD) (HP6890 GC and HP7673) autosampler was used to perform the Gas Chromatography-Mass Spectroscopy (GC–MS) analysis [13] of the components. It was operated at 65eV using acquisition scan mode with HP-5MS at an oven temperature of 100˚ C, held for one minute, and then raised to 280˚ C by 20˚C/min, held for ten minutes. Helium was employed as the carrier gas at a steady column flow rate of 1.5 ml/min, and the injector temperature was set at 250˚C. A 2µl aliquot of the sample extract was injected using the split, less mode approach. Target components were chosen from ten chromatograms, and chromatograms were registered using Comp Extractor software. The text included references to identified compounds and their documented biological activity in Figure 1 and Table 2.

2.5 Ethical Approval : The institutional Animal Ethics Committee (IAEC/V/03/RIPER/2024) has approved the experimental protocol to carry out the research at Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapuramu, Andhra Pradesh, India. Animals Wistar Albino rats of both sexes (200-250 grams body weight) were purchased from Venkateshwara laboratories, Bengaluru for this study. They were housed at temperature (22±10C), relative humidity (55±5% RH) and 12 h light/dark cycles. Animals were allowed to have free rat pellet diet and water was given ad libitum throughout the experiment.

2.6 Evaluation of Hepatoprotective activity:

Acute oral toxicity experiments were conducted for *Parmotrema perlatum* aqueous extracts in accordance with OECD NO: 42011 recommendations provided by the Organization for Economic Cooperation and Development. For the investigation, 200–250 g Wistar albino rats of both sexes were employed. Since there are six animals in each group, these animals were split up into five groups and put to an overnight fast. Animals in all groups received oral administration of an aqueous extract of *Parmotrema perlatum* at a dose of 5 mg/kg . Skin rashes, lacrimation, sneezing, hair loss, and death were among the symptoms of toxicity that were monitored for one hour and then three hours. Since there was no mortality at this dosage, all groups received extracts at doses of 50 mg/kg, 300 mg/kg, and 2000 mg/kg once more. To detect any toxicity or delayed mortality, these animals were monitored for up to 14 days following oral administration of the extract. The OECD standards for oral administration of a single herbal extract acknowledged 2000 mg/kg bw as a non-toxic dose. Choosing a dosage To conduct in vivo antioxidant and hepatoprotective tests, a low dose of 1/10th of the non-toxic maximum dose and a high dose of 1/5th of the non-toxic maximum dose were chosen.[9]

2.7 Experimental design for In vivo study : Thirty Wistar albino rats of both sexes were randomly assigned to five groups, each with six animals, in order to investigate in vivo antioxidant and hepatoprotective effects. The experiment was planned to last 21 days and involved oral administration of a single dose every 24 hours.

Group 1: Normal control rats were received 2 ml of 2% gum acacia (0.1 g/200 g bw) for 21 days.

Group 2: Negative control rats received 2g/kg/day ethanol (40%) for a period of 21 days.

Group 3: Received 1ml of 2% acacia suspension containing 50 mg/kg bw of silymarin as standard along with 2g/kg/day ethanol (40%) as daily dose.

Group 4: Received a solution containing 2g/kg/day ethanol (40%) and 200 mg/kg bw of aqueous extract of *Parmotrema perlatum* for 21 days.

Group 5: Received a solution containing 2g/kg/day ethanol (40%) and 400 mg/kg bw of aqueous extract of *Parmotrema perlatum* for 21 days.

On 22nd day blood samples were collected by retro orbital plexus then transferred into a sterilized centrifuge tube, allowed to clot and serum was separated at 2500 rpm about 15 min to evaluate various biochemical parameters for liver function study by estimation of Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) according to King (1965) method [13], serum bilirubin levels as reported by Malloy et al., (1937)

[12] respectively using the diagnostic kits purchased from Sigma Aldrich (Bengaluru, India)

Following the removal of liver tissue for histological investigations, each animal's remaining liver was rinsed in ice-cold normal saline, then 0.15 M Tris-HCl (pH 7.4), blotted dry, and weighed in order to measure antioxidant activity. After making a 10% w/v homogenate, a few milliliters of trichloroacetic acid (TCA) were added to the precipitated proteins, filtered, and centrifuged for 15 minutes at 4°C at 15000 rpm. Superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels were estimated using the resulting supernatant using the standard procedures outlined by Mishra & Fridovich (1971), Hugo E. Aebe (1979), and Moran M.S. et al. (1979), respectively. [10-11]

3. RESULTS

3.1 Phytochemical Screening:

As shown in Table 1, the phytochemical screening of the *Parmotrema perlatum* aqueous extract had identified the presence of phenolics, flavonoids, tannins, terpenes, alkaloids, glycosides, etc.

Table 1: Table of Phytochemical Tests

Name of secondary metabolite	Identification test	Observation	Inference
Cardiac glycosides	Legals test	+	**Cardiac glycosides are present**
	Keller-Killani Test	+	
	Baljet test	+	
	Kedde's test	+	
Tannins	$FeCl_2$ test	+	**Tannins are present**
	Lead acetate	+	
	PotassiumDichromate	+	
	Iodine Solution	+	
	Nitric acid test	-	
Steroids	Salkowski test	-	**Steroids are present**
	Libermann's test	+	
	Libermann-Buchard test	+	
Alkaloids	Dragendroff's reagent	+	**Alkaloids are present**
	Mayers reagent	+	
	Hager's test	+	
	Tannic acid test	+	
	Wagner's test	_	

3.2 GC-MS Study: The following compounds were identified as shown in the Figure No.1& Table No.2

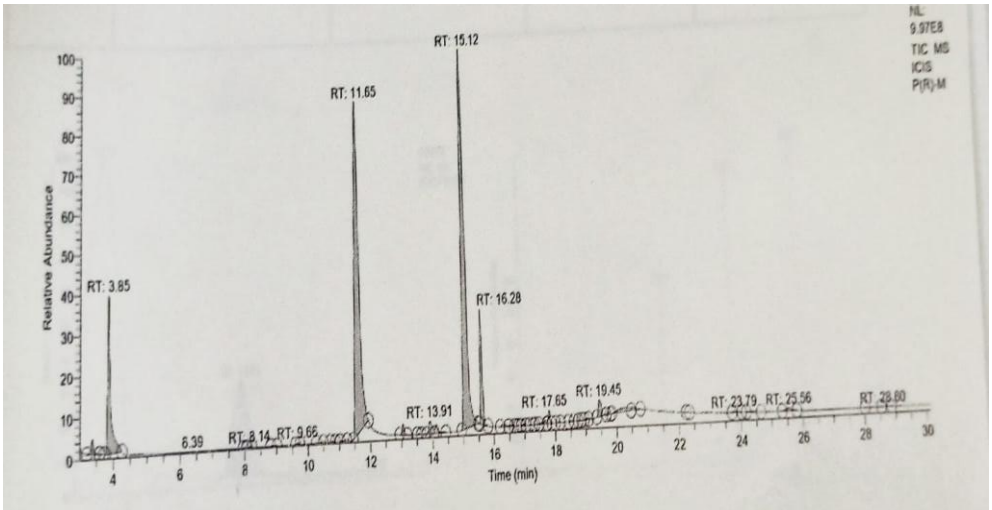

Fig.1:Phytochemical compounds identified in GC-MS Spectrum

Table 2: Compounds identified in GC-MS Spectrum

S.NO	COMPOUND NAME	MOLECULAR FORMULA	RETENTION TIME	PEAK AREA %
1	5,7- dihydroxy 4- methyl coumarin	$C_{10}H_8O_4$	15.62	5.92
2	Platambin-1,6 dione	$C_{15}H_{22}O_2$	19.03	0.08
3	1,2- Longidione	$C_{15}H_{22}O_2$	19.03	0.08
4	Gamolenic acid	$C_{18}H_{30}O_2$	23.79	0.12
5	Sigmastan 3,5-diene	$C_{29}H_{48}$	25.56	0.45

3.3 HISTOPATHOLOGY MICROSCOPY STUDY:

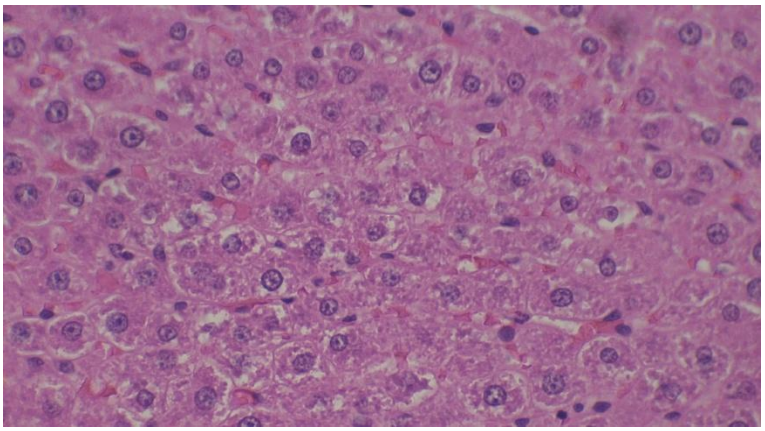

Fig no:2 Histopathology Microscopic Study of Control.

The histological examination of liver tissue obtained from the control group, which was treated with 2% Gum Acacia, revealed a normal hepatic organization. The well-organized hepatocytes in radiating cords or plates encircling the central vein are a feature of good liver histology. The spherical, pronounced nuclei of these cells were easily visible, suggesting normal nuclear morphology and active cellular activity. There was no indication of congestion or dilatation, and the hepatic

sinusoids—the blood spaces between the hepatocyte cords—were clearly visible and uniformly distributed. Importantly, no pathological changes such as cellular infiltration, fatty degeneration, necrosis, fibrosis, or inflammation were seen. This suggests that the administration of 2% Gum Acacia did not induce any toxic or adverse effects on the liver tissue, thereby validating its safety in the control group under the experimental conditions.

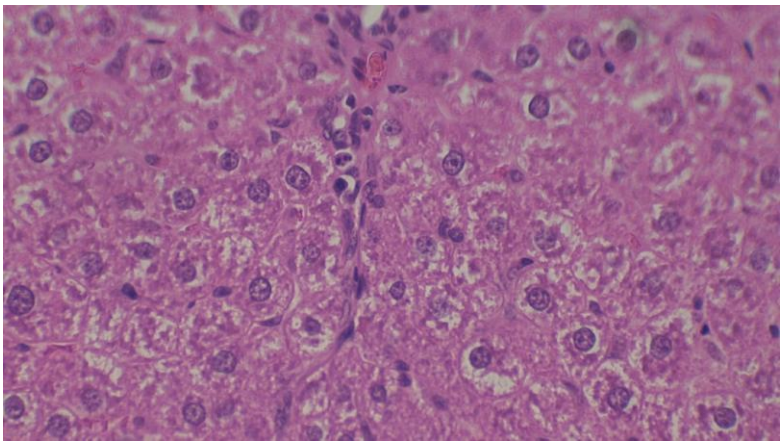

Fig no:3 Histopathology Microscopy Study of Negative control(Ethanol)

The histological examination of liver tissue collected from the negative control group, which was treated with ethanol, revealed severe structural damage indicative of early-stage alcoholic liver injury. The hepatic architecture was disrupted, with significant evidence of alcoholic steatosis—accumulation of lipid droplets inside the hepatocytes, suggesting fatty liver alterations. Hepatocyte necrosis was clearly visible, as evidenced by the fragmentation of cell nuclei and loss of cellular integrity. Additionally, there were inflammatory alterations, including lymphocyte infiltration, which suggested an immunological reaction to liver cell damage. Additionally, a diffused effacement of cellular architecture was observed, disrupting the normal arrangement of the hepatic cord. Proliferation of hepatocytes was noted, potentially as a compensatory mechanism. Additionally, the blood arteries were dilated, which may be related to vascular congestion or inflammation. All of these results support ethanol-induced hepatotoxicity, which is typified by necrosis, inflammation, fatty degeneration, and architectural disarray.

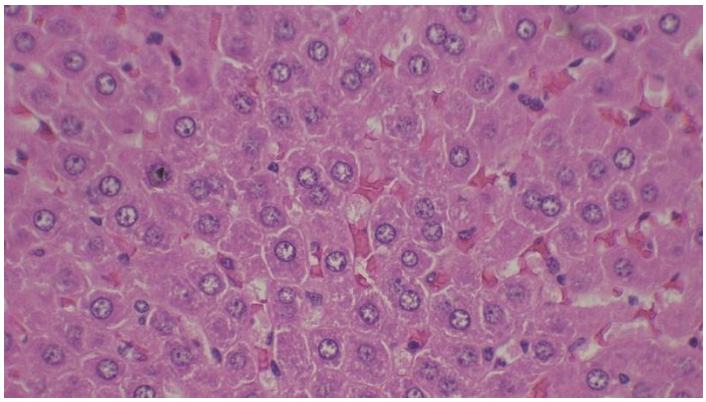

Fig no:4Histopathological Study of Standard(Silymarin)

The histological study revealed a notable improvement in liver architecture between the liver tissue from the group treated with ethanol + silymarin and the group treated with ethanol alone. The hepatic cells' morphology stayed quite close to that of typical liver histology; they were organized in tidy cords, polygonal, and had nuclei in the middle. The sinusoidal spaces were clearly visible, and there was minimal congestion. Inflammatory cell infiltration was either absent or drastically reduced, and there were no overt signs of necrosis or steatosis. The hepatic vasculature showed no signs of disruption or dilation. These findings showed that silymarin has a hepatoprotective effect, significantly reducing ethanol-induced liver damage while preserving normal cellular integrity and tissue organization. The observation of a nearly normal hepatic structure validates silymarin's efficacy as a common hepatoprotective medication in experimental models of liver injury.

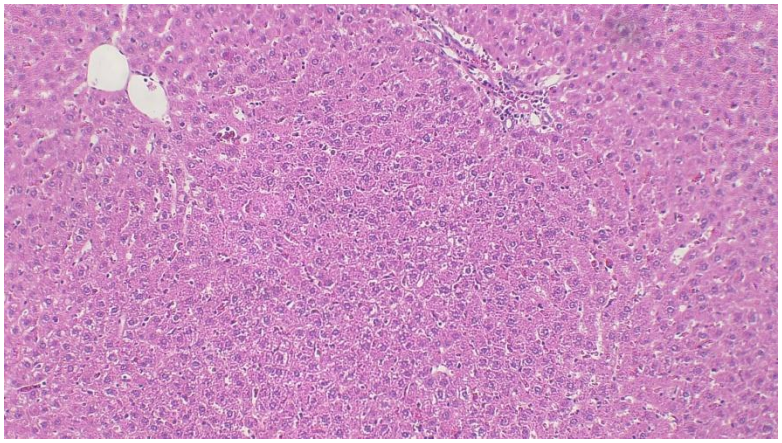

Fig no: 5 Histopathology Microscopy study of low dose

The hepatic lobular architecture is intact when liver tissue treated with a low dose is examined histologically. From the slightly off-center central vein, hepatocytes seem to be arranged in discrete cords or plates. This configuration represents a liver structure that is typically normal. Hepatocytes encircle the visible central vein, suggesting that the integrity of the liver tissue has been preserved. There is mild parenchymal alteration, with minimal necrosis and inflammation observed, suggesting only slight cellular damage. These findings imply that the low dose administration does not induce significant hepatotoxicity and the liver retains most of its structural and functional characteristics

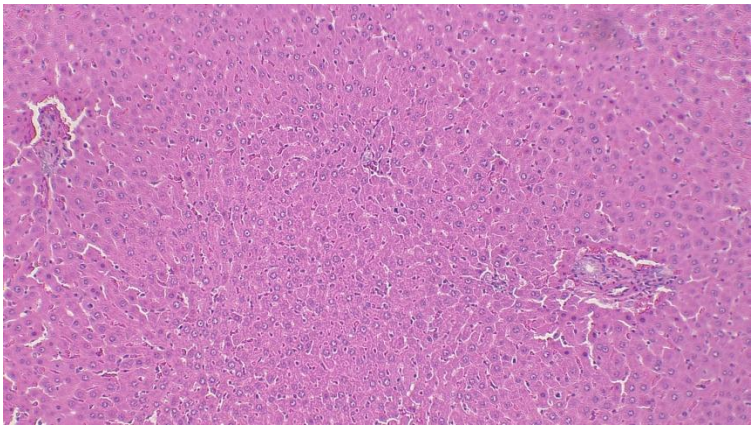

Fig no: 6 Histopathology Microscopy Study of high dose

The hepatic architecture of the liver tissue from the high-dose treatment group was found to be well-preserved upon histological analysis. The hepatocytes showed typical cytoplasmic and nuclear morphology, as well as well-defined cellular borders. Pathological indicators such necrosis, cellular degeneration, or inflammatory cell infiltration were not seen. According to these results, the high dosage of the given substance demonstrated hepatoprotective capacity, preserving the integrity of the liver parenchyma and guaranteeing typical histological characteristics.

4. DISCUSSION

Liver injury is a complex condition that is often brought on by exposure to hepatotoxic medications or xenobiotics, metabolic abnormalities, viral infections, and alcohol intake. The liver is particularly vulnerable to oxidative stress and inflammatory injury because of its pivotal function in detoxifying activities. Natural products have demonstrated great potential in reducing such damage because of their anti-inflammatory, antioxidant, and membrane-stabilizing qualities, particularly those that contain polyphenols like flavonoids.

The hepatoprotective and antioxidant properties of *Parmotrema perlatum*, an edible lichen that has long been employed in Indian food and ethnomedicine, were examined in this study. Cold maceration produced the aqueous extract, which was then subjected to GC-MS analysis and phytochemical screening. Key secondary metabolites such as flavonoids, phenolics, alkaloids, tannins, glycosides, and terpenoids—compounds known for their hepatoprotective qualities—were found to be present by the phytochemical examination.

The medicinal potential of *Parmotrema perlatum* was further validated by GC-MS profiling, which identified a number of bioactive chemicals known to have substantial hepatoprotective and antioxidant properties. By increasing endogenous enzymes such reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), the antioxidant defence was validated in vivo. Reactive oxygen species (ROS), which are essential to alcohol-induced liver damage, are neutralized in large part by these enzymes.

Serum values of SGOT, SGPT, ALP, and bilirubin were markedly raised in the ethanol-treated negative control group, indicating severe liver injury. This is consistent with histological findings of vascular congestion, steatosis, necrosis, and inflammatory infiltration—all of which are indicators of alcoholic liver disease. These results highlight the detrimental effects of extended exposure to ethanol on the structure and function of the liver.

On the other hand, the group that received a low dose of *Parmotrema perlatum* extract (200 mg/kg) demonstrated a significant decrease in serum liver enzymes and a recovery of antioxidant indicators. This group's histopathology showed minor inflammatory or necrotic changes, modest parenchymal abnormalities, and intact liver architecture. These findings suggest that the extract has a moderately protective effect at this dosage.

The aqueous extract showed even more noticeable hepatoprotective activity at the higher dose (400 mg/kg). Histopathological sections showed intact hepatocyte morphology, a well-preserved hepatic lobular structure, and no discernible cellular damage. The biochemical markers also almost returned to normal. These effects were similar to those seen in the group that received regular silymarin treatment, indicating that *Parmotrema perlatum* had strong hepatoprotective potential.

There are multiple processes by which *Parmotrema perlatum* protects the liver:

1. Antioxidant effect through the overexpression of antioxidant enzymes and the scavenging of free radicals.
2. Anti-inflammatory impact through NF-κB signaling and pro-inflammatory cytokine inhibition.

3. Hepatocyte membrane stabilization, which lessens liver enzyme leakage into serum.

4. Cytochrome P450 enzyme modification, which lowers the production of harmful metabolites.

5. Histological preservation, which validates the protective effect seen in biochemical tests.

These results show that *Parmotrema perlatum* is a good option for additional development and are consistent with the pharmacological characteristics of other botanicals rich in flavonoids. Because of its diverse phytoconstituent composition, it could be a source of new hepatoprotective medicines that are less expensive and safer than traditional synthetic medications. Furthermore, the incorporation of these natural compounds into sophisticated drug delivery systems or nutraceuticals may improve patient compliance and therapeutic efficacy.

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