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Ethnomedicinal Survey of Hepatoprotective Plants of Raisen District, Madhya Pradesh, India

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Abstract
Liver disorders represent a huge burden of global health with fewer efficient conventional therapies and significant side effects. Traditional medicine, especially ethnobotanical therapy, is a goldmine for new hepatoprotective drugs. This article describes an extensive ethnomedicinal survey to document, identify, and scientifically prove the plants consumed by local communities and traditional healers in the Raisen District of Madhya Pradesh, India, to treat liver disorders. Raisen, being rich in biodiversity and indigenous knowledge systems, is a perfect geographic location to conduct such a study. The research suggests a protocol including ethical clearance, systematic field survey, semi-structured interview of traditional healers, collection and identification of plants, preliminary phytochemical screening, and in vitro and in vivo pharmacological tests to determine hepatoprotective activity. The anticipated outcomes are the documentation of a wide range of medicinal plants with possible liver protective activities, relationship between traditional knowledge and current scientific information, and determination of promising plant extracts worthy of further isolation of bioactive compounds. This study seeks to give scientific rationale to traditional practices, support drug discovery, and ensure the sustainable exploitation and conservation of medicinal plants in the area.
Keywords: *Ethnomedicine, Hepatoprotective, Raisen District, Madhya Pradesh, Traditional Knowledge, Medicinal Plants, Ethnopharmacology, Liver Disease.*

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I. Introduction

The liver, an essential organ, plays a central role in metabolism, detoxification, protein synthesis, and bile production. Its dysfunction, caused by several factors like viral infections, alcoholism, drug toxicity, autoimmune diseases, and metabolic conditions, results in a range of liver pathologies, ranging from hepatitis to cirrhosis, fatty liver disease, and cancer of the liver [1]. As per the World Health Organization (WHO), liver diseases are responsible for a considerable part of all mortalities worldwide, and their prevalence is increasing further, especially in developing nations [2]. Present-day conventional approaches tend to have serious side effects, are expensive, and in some cases, have limited efficacy, particularly when the liver damage is advanced, necessitating an urgent requirement for safer and more effective therapeutic options [3].

Traditional systems of medicine, with deep ethnobotanical roots, have been delivering primary healthcare for centuries to a significant portion of the world's population. India, which is a mega-biodiversity nation, has a rich tradition of traditional medicine in the forms of Ayurveda, Unani, Siddha, and local folk practices, which make widespread use of medicinal plants for the cure of a vast number of diseases [4]. Such conventional systems are mostly based on a holistic strategy and have been a rich source for finding many of the modern medicines [5]. The Raisen District, which is in the center of Madhya Pradesh, India, is known to be rich in various forest ecosystems, such as deciduous forests, which support a rich diversity of flora and fauna. The area is also home to indigenous people and traditional healers who hold rich ethnomedicinal experience transmitted from generation to generation [6]. Despite sporadic accounts of the overall medicinal flora of Madhya Pradesh, a systematic and specific ethnomedicinal study aimed at hepatoprotective plants in the Raisen District, and subsequent scientific substantiation, is yet to be explored.

The current investigation intends to fill this gap by scientifically documenting the traditional knowledge of the plants used in liver diseases in Raisen District. Additionally, it seeks to give scientific evidence in support of these ethnomedicinal claims using modern pharmacological and phytochemical approaches. This method not only maintains and honors indigenous knowledge but also provides a rational means of uncovering new, effective, and safe hepatoprotectors from nature.

II. Literature Review

Global Burden of Liver Diseases and the Implication for New Therapeutics Liver diseases contribute significantly to morbidity and mortality globally. Viral hepatitis B and C, alcohol liver disease, and non-alcoholic fatty liver disease (NAFLD) are the most common causes of chronic liver disease that may lead to cirrhosis, liver failure, and hepatocellular carcinoma [7]. Even after great strides in medical science, impactful treatments, especially for end stages of liver injury, are still a challenge. Liver transplantation is usually the last option, with the challenge of organ availability, expense, and surgical complications [8]. This situation emphasizes the pressing need for safe, inexpensive, and readily available hepatoprotective treatments, especially of natural origin.

Ethnomedicine as a Drug Discovery Resource Natural products, and mostly from plants, have been a drug discovery cornerstone throughout history. Around 25% of contemporary medicines are directly or indirectly based on plants [9]. Ethnomedicinal research, which entails the organized documentation of conventional medical practices and the associated herbs, is an irreplaceable roadmap for targeted drug discovery initiatives. By working on those plants with prior documented traditional usage for certain diseases, scientists can decrease the lead compound discovery time significantly and enhance the chances of obtaining biologically active compounds [10]. Some of the well-established hepatoprotective drugs, like silymarin from Silybum marianum, originated in folk medicine [11].

Phytochemistry and Mechanism of Hepatoprotection

Medicinal plants achieve their hepatoprotective action through several mechanisms, most of which are related to their rich phytochemical content. Such mechanisms involve:

Antioxidant activity: Minimizing oxidative stress, a principal mediator of liver damage, through free radical scavenging, promotion of endogenous antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione reductase), and prevention of lipid peroxidation [12].

Anti-inflammatory effects: Regulation of inflammatory pathways, inhibition of pro-inflammatory cytokines, and attenuation of immune-mediated liver injury [13].

Anti-fibrotic activity: Prevention or reversal of the build-up of extracellular matrix proteins, causing liver fibrosis and cirrhosis [14].

Apoptosis modulation: Modulation of programmed cell death in hepatocytes, with protection against injury without promoting the death of damaged cells [15].

Amplification of detoxification pathways: Facilitating hepatic detoxifying enzymes (e.g., cytochrome P450 system, glutathione S-transferases) [16]. Flavonoids, phenolic acids, tannins, alkaloids, triterpenoids, saponins, and polysaccharides are common phytochemical classes involved in hepatoprotection [17].

Earlier Studies in Madhya Pradesh Madhya Pradesh, being a state of dense tribal population and varied forest cover, has been targeted by various ethnomedicinal surveys. Various studies from districts have reported the use of plants against fever, skin diseases, gastrointestinal ailments, and to a lesser degree, liver disorders [18, 19]. Most of these studies are, however, general ethnobotanical surveys with no specific emphasis on hepatoprotective activity or subsequent scientific research. A definite requirement exists for focused research in geographic areas such as Raisen, in order to systematically find and confirm putative new drug leads.

III. Materials and Methods

The present study will take a multi-pronged approach incorporating ethnobotanical methods with contemporary pharmacological and phytochemical screening methods.

Study Area -The study will be performed in different forest blocks and villages of the Raisen District of Madhya Pradesh, India. Raisen District is located in the central region of Madhya Pradesh, with its undulating landscape, encompassing portions of the Vindhya Range, as well as a combination of tropical dry deciduous and tropical moist deciduous forests. The district encompasses varied communities, such as those possessing traditional knowledge systems and utilizing local plants for medicinal purposes.

Ethical Clearance -Before initiating any fieldwork with human subjects, ethical clearance will be taken from the Institutional Ethics Committee (IEC) of the concerned affiliating institution. The forest department and local administration of the Raisen District will also be given permission. Informed consent (verbal as well as written, with understanding in local language) will be taken from all those traditional healers and community members who will be participating in the interviews. Due care will be exercised in preserving the intellectual property rights and traditional knowledge of the communities according to the guidelines of the Nagoya Protocol.

Ethnomedicinal Survey and Data Collection

Participant Selection: Traditional healers (Vaidyas, Ojhas, tribal practitioners), senior community members, and experienced individuals with known reputations for utilizing medicinal plants will be selected using snowball sampling and local advice.

Data Collection Tools: Semi-structured interviews, guided field walks, and focus group discussions will be utilized. A pre-structured questionnaire will be utilized to document information, including:
Socio-demographic information of the informants.
Informal name of the plant.
Plant part(s) utilized (leaf, stem, root, bark, fruit, flower, whole plant).
Method of preparation (decoction, infusion, paste, powder, juice).
Dosage and administration route.
Particular liver conditions treated (jaundice, swelling of liver, loss of appetite, general liver weakness, etc.).
Any known side effects or contraindications.
Data Analysis: The collected ethnomedicinal data will be analyzed using quantitative ethnobotanical indices such as Fidelity Level (FL), Relative Frequency of Citation (RFC), and Informant Consensus Factor (ICF) to identify plants with high consensus among healers for liver-related ailments [20].
Plant Collection and Identification
Collection: Samples of plants documented to possess hepatoprotective activity will be harvested from their native environments in the company of traditional healers to ensure accurate identification. Healthy and mature portions of the plant only will be harvested in adequate amounts for further studies.
Authentication: Specimens of vouchers will be prepared in accordance with standard herbarium practices. The plants collected will be identified by a trained botanist and their scientific name, family, and local name noted. Voucher specimens will be stored in an accredited herbarium for future identification.
Phytochemical Screening The authenticated plant materials will be finely washed, shade-dried, and pulverized into a coarse powder. Extracts will be made with different solvents (e.g., ethanol, methanol, water, petroleum ether, chloroform) following traditional preparation and prevailing ethnopharmacological practices.

Preliminary Phytochemical Screening: The extracts are to be subjected to routine qualitative chemical tests for the detection of the presence of major classes of phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids, triterpenoids, carbohydrates, and phenolic compounds [21].
Quantitative Phytochemical Analysis: The extracts with promising activity will be analyzed for total phenolic content (TPC), total flavonoid content (TFC), and other concerned compounds using spectrophotometric assays.
Pharmacological Evaluation of Hepatoprotective Activity
In vitro Studies

Cell Culture: Primary hepatocytes or human hepatocellular carcinoma (HepG2) cell line will be employed.
Cytotoxicity Assay: MTT assay will be used to identify the non-toxic concentrations of the plant extracts.
Drug-induced Hepatotoxicity Models:
Carbon Tetrachloride (CCl4)-induced toxicity: Cells will be co-treated with plant extracts and CCl4 to evaluate protection against oxidative injury.
Paracetamol (APAP)-induced toxicity: Cells will be treated with APAP to cause necrosis, then evaluation of the efficacy of extracts.
Biochemical Markers: Quantification of lactate dehydrogenase (LDH) leakage, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations in supernatants of cell culture.
Antioxidant Assays: DPPH, ABTS, FRAP assays for determination of free radical scavenging activity and reducing power. Determination of intracellular reactive oxygen species (ROS) by DCFH-DA assay [22].
Anti-inflammatory Assays: Determination of pro-inflammatory cytokines (e.g., TNF-α, IL-6) by ELISA in LPS-stimulated macrophages or hepatocytes [23].

3.6.2. In vivo Studies (Animal Models) After taking ethical clearance from the Institutional Animal Ethics Committee (IAEC), high-potential extracts will be tested in in vivo models.

Animal Model: Wistar albino rats or Swiss albino mice are used.
Hepatotoxicity Induction:
CCl4-induced hepatotoxicity: Animals will be given CCl4 intraperitoneally to cause acute or chronic liver damage.
Paracetamol (APAP)-induced hepatotoxicity: Oral administration of high doses of APAP will be given to cause acute liver damage.
Treatment Groups: The animals will be allocated into control, toxicant control, standard drug (e.g., silymarin), and different doses of plant extract treatment groups.
Biochemical Analysis: Serum will be examined for liver function markers: ALT, AST, alkaline phosphatase (ALP), total bilirubin, total protein, albumin. Oxidative stress markers such as malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) in liver homogenates [24].

Histopathological Examination: Liver tissue samples will be obtained, fixed, stained (e.g., H&E), and microscopically examined for evidence of necrosis, inflammation, steatosis, and fibrosis.
Statistical Analysis: Data will be expressed as mean ± standard error of the mean (SEM). Statistical significance will be tested by ANOVA followed by post-hoc tests (e.g., Dunnett's test) with $p < 0.05$ being significant.

Expected Results and Discussion

Ethnomedicinal Documentation The ethnomedicinal survey is likely to document a good number of plant species employed for different liver diseases by the local inhabitants of Raisen District. A good number of formulations, plant parts (usually leaves, roots, or bark), and preparation techniques will be documented. For instance, decoctions of Phyllanthus emblica bark for jaundice or Andrographis paniculata leaves for overall liver debility could be used by local healers, consistent with available scientific literature on these species [25, 26]. ICF and

S. No.	Species	Habit	Part used	Ethno-medicinal uses
1	*Achillea millefolium* L. [Asteraceae];	Herb	Leaves	Fever, diarrhea, toothache, snakebite.
2	*Aconitum heterophyllum* Wall. ex Royle [Ranunculaceae];	Herb	Root	Anti-diarrheal, expectorant, antiinflammatory and UTI.
3	*Acorus calamus* L. [Acoraceae];	Herb	Root	Diarrhea, cough, snake bite,gastrites and flatulence.
4	*Aesculus indica* (Colebr. ex Cambess.) Hook. [Sapindaceae];	Tree	Leaves, seeds	Frostbite.
5	*Arnebia benthamii* (Wall. ex G.Don) I.M.Johnst. [Boraginaceae];	Herb	Whole plant	Enhance lactation, tongue and mouth disease and fever.
6	*Artemisia absinthium* L. [Asteraceae];	Shrub	Leaves, stem	Anti-helminthic, stomach and gallbladder ailments and obesity.
7	*Atropa acuminata* Royle ex Lindl. [Solanaceae];	Herb	Leaves, roots	Cough, abdominal problems, pupil dilation.
8	*Berberis lycium* Royle [Berberidaceae];	Shrub	Roots, fruits	Splenic problems, throat and chest troubles, eye disease jaundice and cholera.
9	*Calendula officinalis* L. [Asteraceae];	Herb	Whole flower	Wound healer, eczema, body rashes and antiseptic.
10	*Cannabis sativa* L. [Cannabaceae];	Herb	Leaves, seeds	Excessive urination, convulsions, tumors and ulcers.
11	*Capsella bursa-pastoris* (L.) Medik. [Brassicaceae];	Herb	Leaves	Continuous bleeding, diuretic.
12	*Celosia argentea* L. [Amaranthaceae];	Herb	Leaves, flowers	Food color, fever, jaundice and diarrhea.
13	*Cuscuta europaea* L. [Convolvulaceae];	Herb	Leaves, stem	Laxative, hair fall.
14	*Dipsacus inermis* Wall. [Caprifoliaceae];	Herb	Whole herb	Bathing.

15	*Dolomiaea costus* (Falc.) Kasana & A. K. Pandey (syn.*Saussurea lappa* (Decne.) Sch.Bip.) [Asteraceae];	Herb	Rhizome	Perfume, rheumatism, scabies and bronchial asthma.

FL values will pick out plants of greatest consensus among healers, reflecting their likely efficacy and ordering these for subsequent scientific study.

Phytochemical Profiling Preliminary phytochemical screening should identify the presence of a broad range of secondary metabolites like flavonoids, tannins, alkaloids, and saponins, commonly linked with hepatoprotective activity. Quantitative analysis will give an idea of the level of these particular compounds present in the most lead-like extracts. For example, polyphenol- and flavonoid-rich extracts are likely to be good antioxidants and hence directly connected with liver protection [12].

Hepatoprotective Effects and Mechanisms

In vitro Experiments: Plant extracts would be expected to decrease significantly liver damage markers (e.g., ALT, AST, LDH) in CCl4 or APAP-stimulated cell lines. Potent antioxidant activity (e.g., high DPPH scavenging, elevated SOD/CAT levels) and anti-inflammatory capacities (decreased TNF-α, IL-6) would then represent the first scientific justification for their traditional use.

In Vivo Studies: In the models of animals, the most active extracts should decrease the increase in serum liver enzymes (ALT, AST, ALP, bilirubin) caused by the hepatotoxic agents. Additionally, they should lower oxidative stress (low MDA, high GSH, SOD, CAT) and markers of inflammation in liver tissue. Histopathological analysis will reveal decreased liver cell necrosis, inflammation, and fatty degeneration in treated groups as compared to the toxicant control, reflecting structural protection of the liver [24]. The pharmacological effects observed will be correlated with the detected phytochemical constituents, which will lend scientific rationalization to the traditional claims.

IV. Discussion

The results of this study will provide valuable information on the ethnopharmacology of hepatoprotective plants from Raisen District. The similarity between the folk uses and scientifically proven pharmacological effects will enhance the validity of traditional knowledge systems. The proof of specific plant species and their bioactive compounds may form the basis for new hepatoprotective drugs from plants. The study will also underscore the need to preserve the Raisen District biodiversity and associated traditional knowledge, which is becoming increasingly vulnerable to deforestation and modernization [27]. Problems in standardization of dosage, toxicity, and effectiveness over a long period of these indigenous medicines will also be addressed, opening up avenues for more studies. This research is anticipated to be a part of the international endeavor in finding new therapeutic compounds from natural products, especially for life-threatening diseases such as liver diseases.

This research proposal on ethnomedicinal investigation of hepatoprotective plants of Raisen District, Madhya Pradesh, has aimed to critically document and scientifically confirm traditional uses for liver disease. By incorporating ethnobotanical survey with modern phytochemical and pharmacological analyses, the study is anticipated to provide potential plant species and their extracts with notable hepatoprotective activity. The expected outcomes are the validation of several traditional remedies, identification of their possible mechanisms of action, and the identification of new bioactive compounds. This study will not only give a scientific basis to the traditional knowledge built up over generations but also add greatly towards discovering new, efficient, and safer drugs for liver ailments. In addition, it will highlight the essential need to conserve the rich biodiversity of the Raisen District and the precious ethnomedicinal heritage of its native populations for posterity. Future research will entail purification and structural elucidation of the active compounds, determination of their exact molecular mechanisms, and preclinical and clinical trialings.

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