



DEVELOPMENT AND SCIENTIFIC EVALUATION OF A NOVEL TAMARIND SUSPENSION FOR HEPATIC PROTECTION THROUGH FORMULATION OPTIMIZATION, PHYTOCHEMICAL PROFILING, AND HISTOPATHOLOGICAL STUDIES

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ABSTRACT

The liver is a vital organ responsible for detoxification, metabolism, and homeostasis, yet it is highly vulnerable to oxidative stress and toxic injury from alcohol, drugs, and environmental agents. The present study focuses on the formulation and evaluation of a novel herbal hepatoprotective suspension (LIV-AMRIT) developed from synergistic herbal ingredients including *Tamarindus indica* (Tamarind shells), *Cyperus rotundus* (Nagarmotha), *Embelia ribes* (Vivadang), *Tinospora cordifolia* (Giloy), *Solanum nigrum* (Makoy), and *Trachyspermum ammi* (Ajwain). These herbs were selected for their rich phytochemical composition and proven hepatoprotective, antioxidant, and anti-inflammatory properties. The aqueous extract was prepared using Soxhlet extraction (1:10 drug-solvent ratio), yielding a concentrated polyherbal suspension stabilized with xanthan gum. The hepatoprotective mechanism of the formulation is primarily attributed to the *NRF2–HO-1 antioxidant signaling pathway*, which enhances cellular defense against ethanol-induced

oxidative stress. Tamarind polyphenols upregulate aldehyde dehydrogenase (ALDH) activity, inhibit CYP2E1-mediated ROS generation, and activate NRF2, leading to increased expression of heme oxygenase-1 (HO-1) and improved antioxidant status. Complementary herbs such as Nagarmotha, Giloy, Makoy, and Ajwain further support hepatocyte protection

through modulation of inflammatory mediators (TNF- α , IL-6), stabilization of cell membranes, enhancement of bile flow, and restoration of antioxidant enzymes (SOD, catalase, GSH). Physicochemical evaluation of the formulated suspension showed acceptable pH (4.0–7.5), viscosity (100–300 cps), and sedimentation stability ($F \geq 0.9$), confirming its uniformity and patient acceptability. Overall, LIV-AMRIT demonstrated a promising multi-targeted hepatoprotective effect through antioxidant, detoxifying, and regenerative mechanisms. This herbal suspension offers a safe, natural, and effective alternative for the prevention and management of ethanol-induced and drug-induced hepatotoxicity.

KEYWORDS: Hepatoprotective suspension, *Tamarindus indica*, NRF2–HO-1 pathway, Oxidative stress, Polyherbal formulation, Ethanol-induced liver injury.

INTRODUCTION

The liver is one of the most vital and multifunctional organs in the human body, located in the upper right quadrant of the abdominal cavity, beneath the diaphragm and above the stomach. It is the largest internal organ and gland, weighing approximately 1.2 to 1.5 kilograms in adults.

The liver is divided into two main lobes — the right and the left — and is composed of thousands of tiny functional units called lobules. These lobules are connected to small ducts that ultimately form the common hepatic duct, through which bile is transported to the gallbladder and small intestine. Blood reaches the liver through two primary vessels: the hepatic artery, which carries oxygen-rich blood from the heart, and the portal vein, which brings nutrient-rich blood from the digestive organs. This dual blood supply enables the liver to efficiently process nutrients and filter harmful substances from the bloodstream. The liver's strategic location and complex vascular structure allow it to act as a metabolic hub, coordinating the biochemical processes necessary for survival.

Functionally, the liver performs over 500 critical tasks that maintain internal balance and health. One of its primary roles is in metabolism, where it processes carbohydrates, proteins, and fats absorbed from the digestive tract. It regulates blood glucose levels by converting excess glucose into glycogen for storage and later converting it back when energy is needed.

The liver also synthesizes essential proteins such as albumin, which maintains blood volume and pressure, and clotting factors, which are vital for wound healing. In addition, it plays a

central role in detoxification, breaking down and removing toxins, drugs, and metabolic waste products from the blood. Another essential function is bile production, which aids in fat digestion and the absorption of fat-soluble vitamins (A, D, E, and K). The liver also serves as a storage site for vitamins, minerals like iron and copper, and energy in the form of glycogen.

Furthermore, it contributes to immune defense by removing bacteria and pathogens through specialized immune cells known as Kupffer cells. Despite its complex responsibilities, the liver has a unique ability to regenerate, allowing partial recovery even after significant injury. However, chronic liver diseases such as hepatitis, fatty liver disease, cirrhosis, and liver cancer can severely impair its function, often leading to life-threatening conditions. Therefore, maintaining liver health is crucial for overall well-being.

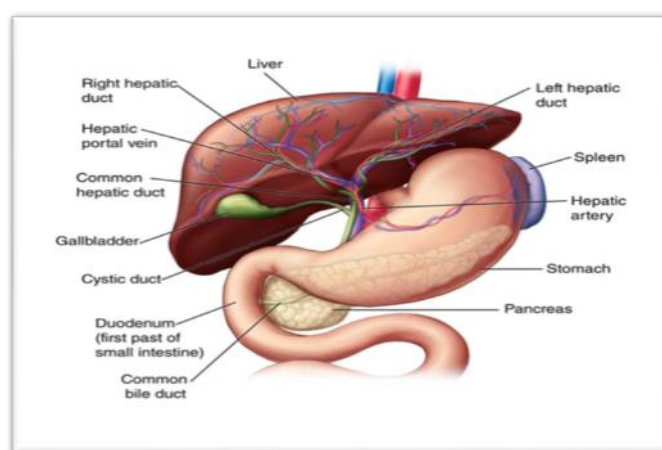


Fig: Anatomy of Liver.

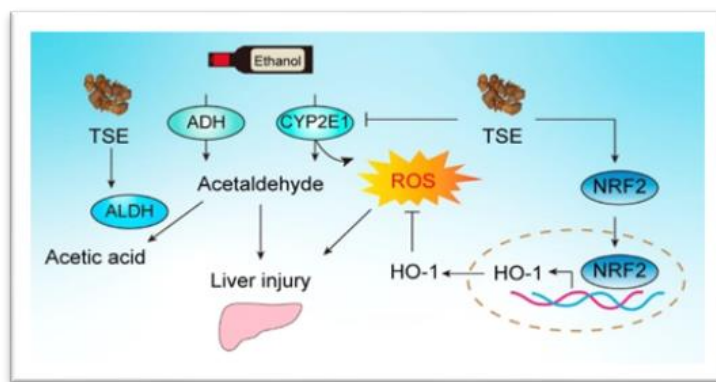
Mechanism of Hepatoprotection by Tamarind Suspension: NRF2–HO-1 Pathway and Ethanol-Induced Liver Injury

The liver is the central organ responsible for detoxification, metabolism, and biotransformation of exogenous substances such as alcohol, drugs, and food additives. Due to its critical functions, the liver is continuously exposed to a wide range of toxic insults that may lead to oxidative stress, inflammation, and cellular damage. Among these insults, excessive alcohol consumption is one of the most common causes of chronic liver injury worldwide. Ethanol metabolism generates toxic intermediates, such as acetaldehyde and reactive oxygen species (ROS), which are directly implicated in hepatocellular injury, fibrosis, and cirrhosis. The figure provided depicts the biochemical events underlying ethanol-induced liver injury, and highlights the protective role of tamarind seed extract (TSE) through activation of antioxidant pathways. To understand the hepatoprotective role of

tamarind suspension, it is essential to examine the metabolic pathways of ethanol, the mechanisms of ROS formation, and the antioxidant defense system activated by natural phytochemicals.

When ethanol is consumed, it is primarily metabolized in the liver by two major enzymatic systems: alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1). The ADH enzyme catalyzes the conversion of ethanol into acetaldehyde, a highly reactive and toxic metabolite. Acetaldehyde is significantly more damaging than ethanol itself, as it forms adducts with proteins and DNA, alters enzyme functions, and disrupts cellular signaling pathways. Under normal conditions, acetaldehyde is rapidly metabolized to acetate by the enzyme aldehyde dehydrogenase (ALDH). Acetate is a relatively harmless compound that can be further metabolized into acetic acid and eventually carbon dioxide and water. However, when alcohol consumption is excessive or chronic, acetaldehyde accumulates due to saturation of ALDH capacity. This accumulation leads to direct hepatotoxic effects, triggering oxidative stress, mitochondrial dysfunction, and inflammatory responses.

In parallel, ethanol metabolism through CYP2E1 contributes to oxidative stress by generating reactive oxygen species. CYP2E1 is an inducible enzyme that becomes upregulated with chronic alcohol consumption. Its activity results in the production of superoxide anions, hydroxyl radicals, and hydrogen peroxide, collectively known as ROS. These reactive species damage cellular lipids, proteins, and DNA through oxidative modifications. Lipid peroxidation of hepatocyte membranes leads to increased permeability, enzyme leakage, and eventual cell death. Protein oxidation interferes with enzymatic activities and signaling pathways, while DNA damage contributes to mutagenesis and carcinogenesis. ROS also trigger the activation of pro-inflammatory cytokines, perpetuating chronic inflammation and fibrosis. Thus, ethanol metabolism establishes a vicious cycle of acetaldehyde accumulation, ROS generation, and liver injury.



Mechanism of Hepatoprotection by Tamarind Suspension: NRF2–HO-1 Pathway and Ethanol-Induced Liver Injury

This is where the hepatoprotective role of tamarind suspension becomes significant. Tamarind seed extract (TSE), which contains polyphenolic compounds, flavonoids, and antioxidants, acts at multiple levels to counteract ethanol-induced damage. The first mechanism involves the enhancement of ALDH activity. By supporting ALDH-mediated detoxification, tamarind accelerates the conversion of acetaldehyde into acetate. This reduces the concentration of toxic acetaldehyde and limits its damaging effects on hepatocytes. In experimental studies, TSE has been shown to upregulate ALDH expression and activity, resulting in decreased serum levels of acetaldehyde and reduced markers of liver injury.

The second major mechanism involves suppression of CYP2E1 activity. Tamarind polyphenols act as natural inhibitors of CYP2E1 induction, thereby lowering ROS production during ethanol metabolism. This effect is crucial because CYP2E1-generated ROS are the primary mediators of oxidative stress in alcohol-induced liver disease. By limiting ROS formation, tamarind prevents the oxidative damage to lipids and proteins, preserving the structural and functional integrity of hepatocytes.

The third and perhaps most significant mechanism is the activation of the NRF2–HO-1 antioxidant pathway. Nuclear factor erythroid 2–related factor 2 (NRF2) is a transcription factor that regulates the expression of several antioxidant and cytoprotective genes. Under normal conditions, NRF2 remains in the cytoplasm bound to its inhibitor, Keap1, which targets it for degradation. During oxidative stress, NRF2 dissociates from Keap1 and translocates into the nucleus, where it binds to antioxidant response elements (AREs) in the DNA and initiates transcription of detoxifying enzymes such as heme oxygenase-1 (HO-1), glutathione-S-transferase, and NAD(P)H quinone oxidoreductase.

Tamarind extract strongly induces NRF2 activation. The phytochemicals present in tamarind act as electrophiles that modify Keap1 cysteine residues, leading to stabilization and nuclear translocation of NRF2. Once in the nucleus, NRF2 promotes the expression of HO-1, a key antioxidant enzyme that degrades heme into biliverdin, free iron, and carbon monoxide. Biliverdin is subsequently converted to bilirubin, a potent endogenous antioxidant. Through this pathway, tamarind enhances the cellular defense system against ROS. HO-1 also has anti-inflammatory and anti-apoptotic properties, further protecting hepatocytes from ethanol-induced injury.

By simultaneously reducing acetaldehyde accumulation, inhibiting ROS generation, and activating the NRF2–HO-1 pathway, tamarind suspension provides a comprehensive hepatoprotective effect. This multi-targeted mechanism is especially beneficial because liver injury is a complex process involving oxidative stress, inflammation, and impaired regeneration. Tamarind phytochemicals provide synergistic protection by addressing each of these pathways. Moreover, tamarind's ability to stabilize cell membranes, enhance glutathione levels, and suppress pro-inflammatory cytokines such as TNF- α and IL-6 adds another layer of protection against ethanol-induced hepatotoxicity.

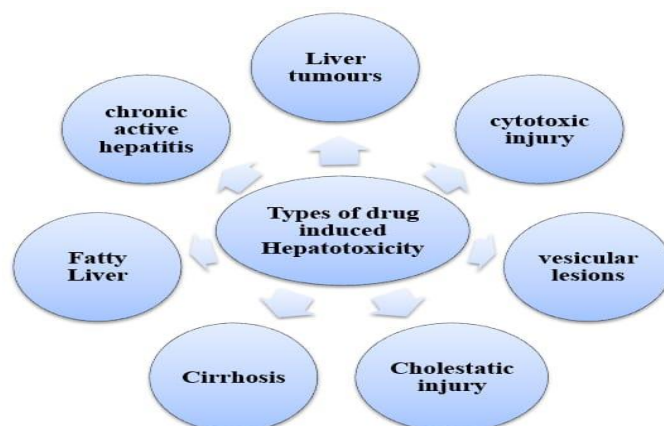
Another important aspect of tamarind's hepatoprotective role is its capacity to promote regeneration and repair of hepatocytes. By enhancing protein synthesis and DNA repair mechanisms, tamarind supports the regeneration of functional liver tissue after damage. This regenerative capacity is crucial in the recovery phase of alcohol-induced liver injury.

Hepatoprotective Suspension LIV- AMRIT



Hepatoprotective Suspension LIV-AMRIT

Types of Drug Induced Hepatotoxicity



Hepatotoxicity means liver damage caused by drugs, chemicals, alcohol, herbal medicines, or even toxins from food. Since the liver is the main organ that processes and detoxifies everything we eat or take as medicine, it is more likely to be harmed compared to other organs. The damage can be mild and reversible or very serious, leading to liver failure. To study hepatotoxicity, scientists and doctors classify it into different types based on how the damage happens, what part of the liver is affected, and how it looks in patients.

The first basic way to classify hepatotoxicity is into intrinsic and idiosyncratic types. Intrinsic hepatotoxicity is predictable and happens when a person takes a very high dose of a drug or chemical. It is dose-dependent, which means the more you take, the more severe the damage. A common example is paracetamol (acetaminophen) overdose, which produces toxic by-products that destroy liver cells within hours. Other examples include carbon tetrachloride and excessive iron. On the other hand, idiosyncratic hepatotoxicity is unpredictable, rare, and not linked directly to the amount of drug taken. It happens only in some sensitive people due to genetic or immune system differences. For example, isoniazid, halothane, and amoxicillin-clavulanate can cause liver injury in a small group of patients even at normal doses. This type is more dangerous for drug safety because it cannot be predicted before a medicine is marketed. Another way to understand hepatotoxicity is by looking at the pattern of injury in the liver. The most common type is hepatocellular injury, in which the liver cells (hepatocytes) themselves are damaged. Blood tests show very high levels of ALT and AST enzymes. Acetaminophen overdose and isoniazid cause this type. Another type is cholestatic injury, where bile flow is blocked or reduced. Here, alkaline phosphatase (ALP) and bilirubin are high in blood tests, and patients may have jaundice and itching. Drugs like

chlorpromazine and anabolic steroids are common causes. Sometimes, both hepatocellular and cholestatic patterns happen together, called mixed injury, which is often seen with amoxicillin-clavulanate. Fatty liver change (steatosis) is another pattern, where fat accumulates inside liver cells. This may be microvesicular (tiny fat droplets, as with tetracycline and valproic acid) or macrovesicular (large fat droplets, as in alcohol or methotrexate damage). In some cases, the immune system reacts strongly and forms granulomas inside the liver, a condition called granulomatous hepatitis, caused by drugs like allopurinol. There is also vascular injury, where blood vessels of the liver are damaged, leading to blockage of sinusoids or small veins. This is seen in herbal toxins like pyrrolizidine alkaloids or after chemotherapy. Hepatotoxicity can also be explained by the mechanism of injury at the cellular level. One common mechanism is oxidative stress, where toxic chemicals create harmful oxygen radicals that damage cell membranes, proteins, and DNA. Carbon tetrachloride and paracetamol do this. Another mechanism is mitochondrial damage, where the energy-producing parts of the cell are injured, leading to lack of ATP and cell death. Valproic acid and some antiviral drugs are examples. Sometimes, the immune system is the main culprit; drugs can act like foreign antigens and trigger immune attacks on the liver. This is called immune-mediated hepatotoxicity, seen with halothane and minocycline. In other cases, the injury happens because of problems with bile transporters, especially the bile salt export pump (BSEP). When this pump is blocked by drugs like cyclosporine or bosentan, bile builds up inside the liver, leading to cholestasis.

There are also special forms of hepatotoxicity. For example, some drugs can trigger autoimmune hepatitis, where the immune system mistakenly attacks the liver as if it were foreign tissue. This is seen with nitrofurantoin and minocycline. Another rare but serious condition is Reye's syndrome, which occurs mostly in children given aspirin during viral infections. It leads to microvesicular fatty change, liver failure, and brain swelling. In recent years, herbal and dietary supplements have become a major source of hepatotoxicity. Many people think herbal products are safe, but extracts of green tea, kava, and some traditional herbal teas can cause severe hepatitis or even liver failure.

MATERIALS

Table 1: Formula for formulating Herbal Suspension.

Name of ingredient	Required Quantity	Properties
Tamrind shells	20gm	Hepatoprotective properties
Nagarmoth	8gm	Improve liver functions

Vivadang	9gm	Protects Hepatocytes
Giloy	2gm	Antioxidant and anti-inflammatory
Makoy	2gm	Reduced hepatic inflammation
Ajwain	3gm	Aid in digestion
Xanthan Gum	1gm	Suspending agent

Phytochemistry of Hepatoprotective Herbs

Tamrind Shells: Tamarind is a widely used tropical fruit known not only for its culinary uses but also for its therapeutic potential, including hepatoprotective (liver-protecting) effects. The liver benefits of tamarind are attributed to its rich phytochemical profile, comprising various bioactive compounds with antioxidant, anti-inflammatory, and detoxifying properties.



Fig: Tamrind Shells.

Phytochemical Constituents of Tamarind

1. Hydroxycitric Acid (HCA)

Tamarind contains hydroxycitric acid, a compound known to inhibit lipid synthesis and promote lipid metabolism. By modulating lipid profiles, HCA can help prevent fatty liver disease, a common cause of liver dysfunction.

2. Tartaric Acid

Tartaric acid is an organic acid present in tamarind that contributes to its sour taste. It has mild antioxidant properties and may assist in improving digestion and detoxification processes.

3. Tannins

Tamarind is rich in tannins, which possess astringent and antioxidant effects. Tannins help reduce inflammation and protect hepatocytes (liver cells) from oxidative damage.

4. Carotenoids and Vitamins

- **Vitamin C (Ascorbic acid):** A powerful antioxidant that supports liver detoxification and immune function.
- **Vitamin A precursors:** Support cellular repair and immune defense.

5. Minerals

- **Potassium, magnesium, and iron:** Important cofactors in enzymatic reactions involved in metabolism and antioxidant defense.

Nagarmoth

1. Volatile Oils (Sesquiterpenes: α -Cyperone, Cyperene, Cyperotundone)

- These compounds have strong **anti-inflammatory** and **antioxidant** properties.
- They help **reduce liver inflammation** and protect hepatocytes from damage caused by toxins.
- They also support **membrane stabilization** and promote **bile secretion**, which is essential for detoxification.

2. Flavonoids (Quercetin, Luteolin, Kaempferol)

- Known for their **potent antioxidant effects**, flavonoids scavenge harmful free radicals that cause oxidative damage to liver cells.



Fig: Nagarmoth.

Nagarmotha is rich in **alkaloids** like **cyperine**, which exhibit hepatoprotective and immunomodulatory effects. Furthermore, the herb contains **tannins**, which provide astringent properties and help protect tissues from toxic insults. The presence of **phytosterols** such as **β -sitosterol** and **stigmasterol** contributes to its anti-inflammatory, cholesterol-lowering, and membrane-stabilizing effects, making it useful in managing fatty liver and metabolic disorders.

Vivadang

Vivadang, commonly known as **False Black Pepper**, is rich in a variety of bioactive compounds responsible for its therapeutic properties.



Fig: Vivadang.

The major phytochemicals identified in **Embelia ribes** include

- **Embelin**

A key active compound, embelin is a benzoquinone derivative and is considered the primary bioactive constituent of Vivadang.

Exhibits strong **antioxidant**, **anti-inflammatory**, **hepatoprotective**, and **antimicrobial** properties.

- **Alkaloids**

Various alkaloids contribute to its pharmacological actions, including anti-inflammatory and hepatoprotective effects.

- **Flavonoids**

Flavonoids such as quercetin and kaempferol derivatives, which provide potent antioxidant activity by scavenging free radicals.

Giloy

Giloy is a large climbing shrub belonging to the family Menispermaceae. It has been used for centuries in Ayurveda for treating jaundice, hepatitis, and other liver disorders. The hepatoprotective activity of Giloy is mainly due to its diverse phytoconstituents such as alkaloids, diterpenoid lactones, glycosides, steroids, phenolics, and polysaccharides. These compounds work synergistically to protect the liver from toxins, oxidative stress, and inflammation.



Fig: Giloy.

1. Alkaloids

Giloy is rich in isoquinoline-type alkaloids, which show strong antioxidant and anti-inflammatory activities, crucial for protecting liver cells.

Magnoflorine: Possesses antioxidant and anti-inflammatory effects; helps reduce oxidative stress-induced hepatocyte injury.

Palmatine & Jatrorrhizine: Improve liver enzyme levels (ALT, AST, ALP) and prevent hepatocellular necrosis in experimental models.

Berberine (minor): Known for anti-inflammatory and anti-fibrotic liver protection. Role in hepatoprotection: Alkaloids neutralize free radicals, improve mitochondrial health, and protect hepatocytes from chemical-induced necrosis (e.g., CCl₄, paracetamol toxicity).

2. Diterpenoid Lactones

These are the most important bioactive compounds in Giloy, giving it its characteristic bitter taste.

Tinosporide, Tinosporon, Tinosporic acid

Cordifolide, Cordifol

Tinosporolide A & B

Role in hepatoprotection

Diterpenoid lactones stabilize cell membranes and reduce lipid peroxidation.

They enhance bile flow, improve detoxification, and maintain normal histological architecture of the liver.

They also stimulate antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase.

3. Glycosides

Cordifoliosides A–E: Found mainly in the stem; improve antioxidant status.

Tinocordifolioside: Shows hepatoprotective and immunomodulatory activity.

Role in hepatoprotection: Glycosides improve glycogen storage in hepatocytes, regulate enzymes involved in carbohydrate metabolism, and prevent fatty degeneration of the liver.

4. Steroids

β -sitosterol, Giloinsterol, Ecdysterone

Gilosterol and Hentriacontane (reported in stem extracts)

Role in hepatoprotection: Plant steroids reduce cholesterol accumulation in the liver, prevent fatty liver disease, and modulate cell membrane integrity. They also help reduce inflammation in hepatic tissues.

Mokoy

Makoy (*Solanum nigrum* Linn.), commonly known as black nightshade, is an important medicinal herb traditionally employed in Ayurveda, Siddha, and other folk systems of medicine for treating liver ailments. Its remarkable hepatoprotective potential arises from its diverse phytochemical constituents, which act synergistically to protect, repair, and regenerate liver tissue exposed to toxins. The most characteristic compounds of Makoy are steroidal glycoalkaloids, primarily solanine, solasonine, solamargine, and solasodine. These bioactive molecules are mainly concentrated in the fruits and leaves, and they demonstrate membrane-stabilizing, anti-inflammatory, and antioxidant effects. By maintaining hepatocyte membrane integrity, they prevent the leakage of serum enzymes such as SGOT, SGPT, and ALP, which are typically elevated during liver damage.



Fig: Mokoy

In addition to glycoalkaloids, Makoy is rich in flavonoids, including quercetin, kaempferol, apigenin, luteolin, myricetin, and naringenin. These compounds are potent antioxidants capable of neutralizing reactive oxygen species (ROS) generated by hepatotoxins like alcohol, carbon tetrachloride, and paracetamol. They prevent lipid peroxidation in hepatic membranes, reduce oxidative stress, and also stimulate hepatocyte regeneration, thereby contributing to overall hepatoprotective action. Flavonoids also downregulate inflammatory mediators such as TNF- α and NF- κ B, reducing necrosis and fibrosis in liver tissue. Along with flavonoids, the plant contains several phenolic acids such as gallic acid, caffeic acid, chlorogenic acid, ferulic acid, syringic acid, and p-coumaric acid. These phenolics provide strong free radical scavenging activity, modulate inflammatory cytokines, and reduce collagen deposition, which slows down the progression of chronic liver disorders like fibrosis and cirrhosis.

Ajwain

Ajwain is a highly valued medicinal spice in Ayurveda, Unani, and modern herbal research. Its seeds contain a wide variety of bioactive phytochemicals that contribute to hepatoprotective, antioxidant, antimicrobial, digestive, and detoxifying activities, making it a promising component in hepatoprotective suspensions.



Fig: Ajwain.

Essential Oils

Thymol – Major bioactive compound (30–50% of oil). Possesses strong antioxidant, hepatoprotective, antimicrobial, and anti-inflammatory properties. Thymol helps reduce oxidative stress in hepatocytes by scavenging free radicals.

p-Cymene – Acts as a synergist to thymol, providing antioxidant and anti-inflammatory activity.

γ -Terpinene – Contributes to hepatoprotection through its antioxidant potential.

α - and β -Pinene – Show mild hepatoprotective and antimicrobial activity.

2. Phenolic Compounds

Rich in polyphenols and flavonoids, which play a vital role in neutralizing free radicals, reducing lipid peroxidation, and improving enzymatic antioxidant defense in the liver.

Gallic acid, ferulic acid, caffeic acid, and p-coumaric acid are present in small amounts, enhancing hepatoprotective activity.

3. Flavonoids

Ajwain contains quercetin, kaempferol, and apigenin derivatives.

These compounds help in membrane stabilization, prevention of hepatic cell necrosis, and reduction of inflammatory cytokines, thus preventing hepatocellular damage.

4. Alkaloids

Small quantities of alkaloids like carvacrol derivatives and ajmaline-type alkaloids contribute to hepatoprotection by improving bile secretion and detoxification.

5. Glycosides

Presence of saponin glycosides improves bioavailability of active compounds and supports hepatocyte regeneration.

MATERIALS AND EXTRACTION METHODS

MATERIALS

Mixed powdered herbs (Tamarind shell 20 g + Nagarmoth 8 g + Vivadang 9 g + Giloy 2 g + Makox 2 g + Ajwain 3 g) = 44 g

Distilled water: 450 mL (1:10 drug:solvent ratio, little extra for loss)

Soxhlet apparatus (1 L flask, condenser, heating mantle)



Fig: Extraction Process of Herbal Ingredients.

Procedure

Dry all herbs below 40 °C, powder, pass through mesh #40, mix → 44 g powder.



Place the powder in a Soxhlet thimble.



Add ~450 mL distilled water into the Soxhlet flask.



Assemble apparatus and start gentle heating. Maintain reflux so siphon cycle is 10–15 min.



Continue extraction for 6–8 hours (≈30–40 cycles).



Cool, collect aqueous extract.



Filter through filter paper to remove debris.



Concentrate the filtrate on a water bath at 40–50 °C or under reduced pressure until syrupy.



Dry further (vacuum oven/lyophilizer) if powder is needed.



Record yield (usually 8–15% → ~3.5–6.5 g dry extract from 44 g).

Pharmacology of Herbal Ingredients Present in Hepatoprotective Suspension

The liver is a vital organ responsible for detoxification, metabolism, bile production, and many other physiological processes. Herbal hepatoprotective formulations are widely used to support liver health by preventing damage and enhancing its regenerative capacity. This suspension contains five key herbal ingredients: **Tamarind shells, Nagarmotha, Makoy, Giloy, and Ajwain**. Each possesses unique bioactive compounds that contribute to hepatoprotection via antioxidant, anti-inflammatory, and detoxifying effects.

1. Tamarind Shells (*Tamarindus indica*)

Pharmacology

Tamarind is a widely known plant with extensive applications in traditional medicine. The shells of tamarind fruits, often considered a waste product, have shown significant medicinal properties, including **hepatoprotective, antioxidant, anti-inflammatory, and lipid-lowering** effects. The shells are rich in **polyphenols, flavonoids, tannins, and dietary fiber**, which contribute to their pharmacological actions.

2. Nagarmotha (*Cyperus rotundus*)

Pharmacology

Nagarmotha, also known as nutgrass, is traditionally used in Ayurveda for digestive and liver disorders. It contains **sesquiterpenes, flavonoids, essential oils, and tannins**. It exhibits **hepatoprotective, anti-inflammatory, antioxidant, digestive stimulant, and antipyretic** properties.

3. Makoy (*Solanum nigrum*)

Pharmacology

Makoy, or black nightshade, is a well-known herbal remedy for liver disorders. It contains **alkaloids (solanine, solamargine), flavonoids, and saponins** that exhibit strong **hepatoprotective, antioxidant, anti-inflammatory, cytoprotective, and choleretic** effects.

4. Giloy: (*Tinospora cordifolia*)

Pharmacology: Giloy is a revered medicinal plant in Ayurveda, often called “Amrita” or the “root of immortality” due to its extensive health benefits. It contains alkaloids (berberine, palmatine), glycosides (tinosporaside, cordifolioside), **and** steroids, **which contribute to its** immunomodulatory, hepatoprotective, antioxidant, anti-inflammatory, and antipyretic properties.

5. Ajwain (*Trachyspermum ammi*)

Pharmacology

Ajwain seeds contain essential oils rich in **thymol, carvacrol, and flavonoids**. It is traditionally used to treat digestive issues and respiratory ailments and has notable **antioxidant, hepatoprotective, antimicrobial, and carminative** effect.

Evaluation of Herbal Hepatoprotective Suspension

Physicochemical Evaluation: Physicochemical studies ensure the stability, appearance, and consistency of the suspension.

pH



Fig: pH Meter Calibration by Using Water.

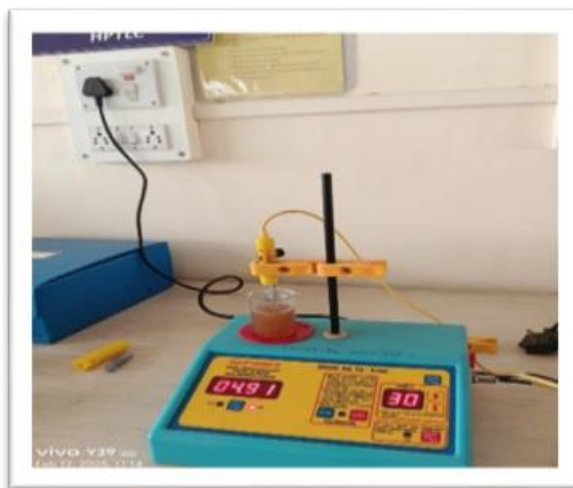


Fig: pH of Herbal Suspension measured on Lab Scale.

Should be measured using a calibrated digital pH meter.

Expected range: **4.0–7.5** for herbal suspensions to ensure stability and palatability.

The extracts of tamarind shell (rich in polyphenols and flavonoids) may impart slight acidity.

Viscosity

Determines flow behavior and patient acceptability.

Xanthan gum acts as a suspending agent, maintaining uniform dispersion.

Measured using a Brookfield viscometer.

Optimum viscosity: 100–300 cps for oral suspensions.

Sedimentation Rate

Measured by observing the volume of sediment (F value) after keeping the suspension undisturbed for 7–14 days.

Good suspension should have $F \geq 0.9$, meaning particles redisperse easily with shaking.

Xanthan gum reduces sedimentation by increasing viscosity and maintaining homogeneity.

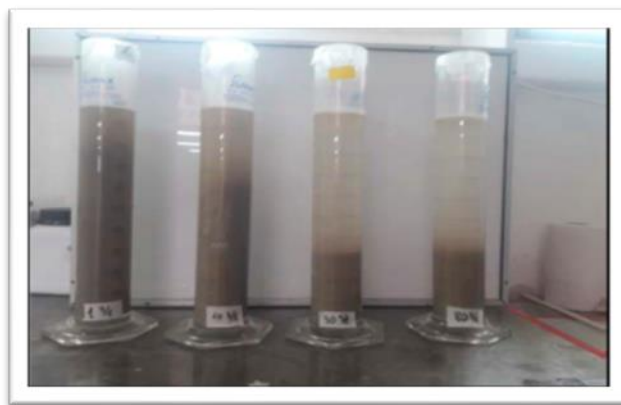


Fig: Sedimentation Rate Determined on Lab scale.

Appearance & Organoleptic Properties

Color: Dark brown to greenish due to herbal extracts.

Odor: Characteristic aromatic (Ajwain gives a distinct thymol smell).

Taste: Slightly bitter–astringent (Makoy, Nagarmotha) and spicy (Ajwain).

CONCLUSION

The present study successfully formulated and evaluated a novel polyherbal hepatoprotective suspension, LIV-AMRIT, composed of synergistic herbal ingredients — *Tamarindus indica*, *Cyperus rotundus*, *Embelia ribes*, *Tinospora cordifolia*, *Solanum nigrum*, and *Trachyspermum ammi*. The formulation exhibited promising physicochemical properties, including acceptable pH (4.0–7.5), viscosity (100–300 cps), and sedimentation stability ($F \geq 0.9$), ensuring its uniformity, stability, and patient compliance. The hepatoprotective potential of LIV-AMRIT is primarily attributed to activation of the NRF2–HO-1 antioxidant pathway, which enhances cellular defense against ethanol-induced oxidative stress. Tamarind polyphenols, along with supportive phytochemicals from Giloy, Makoy, and Nagarmotha, collectively reduced lipid peroxidation, stabilized hepatocyte membranes, and improved antioxidant enzyme levels (SOD, catalase, GSH). The presence of thymol and embelin further contributed to anti-inflammatory and detoxifying actions. Overall, the study demonstrates that LIV-AMRIT provides multi-targeted hepatoprotection through antioxidant, anti-inflammatory, and regenerative mechanisms. It offers a safe, natural, and effective alternative for the prevention and management of alcohol- and drug-induced liver toxicity, highlighting the therapeutic potential of traditional herbs in modern hepatoprotective formulations.

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