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Evaluation of Hepatoprotective and Anti-Hepatotoxicity Activities of Herbal Plants

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ABSTRACT

The body's many physiological functions depend on the liver, which is the most significant organ in this regard. The presence of inflammatory cells in the liver's tissue distinguishes hepatitis, an inflammation of the organ. kinds A, B, C, D, and E relate to the five basic kinds of viruses. The weight of disease and mortality is particularly concerning for these five categories. A serious health issue that affects the pharmaceutical business, drug regulatory organisations, and health care providers is liver damage or malfunction. For a very long time, liver illness has been treated using herbal medications. The body's immune system is the organ that makes diagnoses the infection by activating immune system cells, chemokines, and cytokines, as well as releasing an inflammatory mediator, in order to show an instant reaction. The immune system is strengthened and modulated by them. Polysaccharides, proteins, flavanoids, lignans, and other phytoconstituents originating from plants sustain liver disorders and boost the immune system. Numerous herbs that have been reported to be immunomodulatory and hepatoprotective. The goal of the current review is to gather information on potential phytochemicals found in hepatoprotective and immunomodulatory plants.

Keywords:- Hepatoprotective herb, immunomodulatory herb, nitric oxide

INTRODUCTION

Medicinal plants existing even before human being made their appearance on the earth. Medicinal plants, in India have been collected from the wild and cultivated for millennia. The Rig-Veda, written in India between 4800 and 1600 BC, is the earliest recorded in India for the use of trees, shrubs, herbs and grass combination in curing ailments[1]. According to WHO, 80% of the world population uses plant based remedies as their primary form of healthcare. Throughout the human history people have relied on natural products and plants in a particular, to promote and maintain good health and to fight sickness, pain and disease[2]. The use of herbal medicines is evidence to approach for the treatment and prevention of disease is known as phytotherapy flourishing the quest for significant source of synthetic and herbal drugs[3].

Nowadays herbal products are symbol of safety as when compare to the synthetic products they are unsafe to human life and environment. Now the era of natural products started with the hope of safety and security. Over three-quarter of people in the world depend upon plant extracts to maintain their good health. Of the 2, 50,000 higher plant species on the earth, more than 80,000 are of medicinal values. Indian biodiversity centers contains over 45,000 different plants species, of these, about 15,000 – 20,000 plants have good medicinal value. Among those only 7000 – 7500 plant species are used for their medicinal properties by traditional communities.

In India, Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major system of indigenous medicines since ancient time. Among those Ayurveda describes 700 species, Unani 700 species, Siddha 600 species, Amchi 600 species and Modern Medicine around 30 species. The *Rigveda* (5000 BC) has described 67 medicinal plants, *Yajurveda* 81 species, *Atharvaveda* (4500- 2500 BC) 290 species, *Charaka Samhita* (700 BC) and *Sushruta Samhita* (200 BC) had described 1100 and 1270 species respectively, in compounding of drugs, these are still used in the classical formulations.[4]

About liver, functions and diseases

Liver is the largest glandular organ in the body contributing about 1/50th of the total body weight, which regulates various important metabolic functions and performs many other functions. Liver is also known by a Greek word "*Hepato".* It lies in the upper right quarter of the abdominal cavity. It is reddish- brown in colour and divided into four lobes of unequal sizes and shape.[5] The lobes of the liver are made-up of small lobules, just visible to the naked eye. The lobules are hexagonal from outside and are formed by cubical shaped cells which are hepatocytes and are arranged in pairs of columns radiating from central vein. The sinusoids (blood vessels with an incomplete walls) containing a mixture of blood from the very small branches of the portal vein and hepatic artery. Their arrangement allows the arterial blood and venous blood (with a high concentration of nutrients) to mix and come into close contact with liver cells. The posterior surface of the liver is called portal hepatic, where various structures enter and leave the gland (Figure.1 and Figure. 2)**.** The portal vein enters carrying blood from the stomach, spleen, pancreas and the small and large intestines. The hepatic artery enters, carrying arterial blood, is a branch from the celiac artery, which is a branch from the abdominal aorta. The right and left hepatic ducts leave, carrying bile form the liver to the gall bladder[6].

"*Hepato".* It lies in the upper right quarter of the abdominal cavity. It is reddish- brown in colour and divided into four lobes of unequal sizes and shape.[5] The lobes of the liver are made-up of small lobules, just visible to the naked eye. The lobules are hexagonal from outside and are formed by cubical shaped cells which are hepatocytes and are arranged in pairs of columns radiating from central vein. The sinusoids (blood vessels with an incomplete walls) containing a mixture of blood from the very small branches of the portal vein and hepatic artery. Their arrangement allows the arterial blood and venous blood (with a high concentration of nutrients) to mix and come into close contact with liver cells. The posterior surface of the liver is called portal hepatic, where various structures enter and leave the gland (Figure.1 and Figure. 2)**.** The portal vein enters carrying blood from the stomach, spleen, pancreas and the small and large intestines. The hepatic artery enters, carrying arterial blood, is a branch from the celiac artery, which is a branch from the abdominal aorta. The right and left hepatic ducts leave, carrying bile form the liver to the gall bladder.

Functions of the liver7,8

Liver cells called as hepatocytes are responsible for many functions that are pivotal to normal functioning of human body and they can be basically divided into 3 categories.

a. Regulation, synthesis and secretion.

b. Storage.

c. Purification, transformation and clearance.

a. Regulation, Synthesis and Secretion:

Hepatocytes are helpful for the regulation, synthesis and secretion of many substances important in maintaining the body's normal state.

- Hepatocytes are metabolically active cells those uptake glucose, minerals and vitamins from portal and systemic blood and store them.

- Hepatocytes can produce blood clotting factors, I, II, V, VII, IX, XI, antithrombin, transporter proteins, cholesterol and bile components.

- Regulating blood levels of cholesterol and glucose.

- In the first trimester, fetus in pregnancy, the liver is the main site for RBC production.

b. Storage:

The liver is designed to store important substances such as glucose (in the form of glycogen), fat-soluble vitamins (vitamin A, D, E, and K), folate, vitamin B12 and minerals, such as copper and iron.

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c. Purification, Transformation and Clearance:

The liver plays a major role in removal of waste products, biotransformation of drugs and toxins to less harmful compounds in the blood.

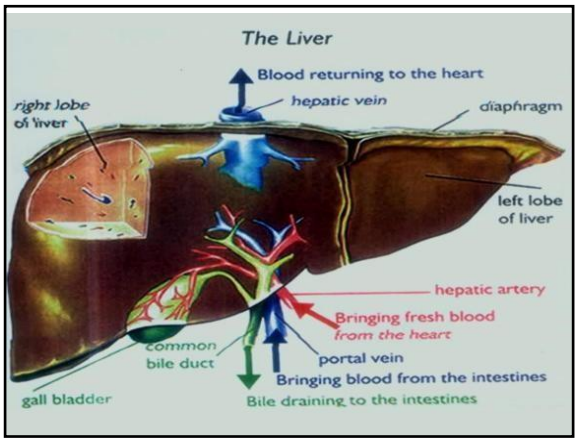

Fig 1. The Liver

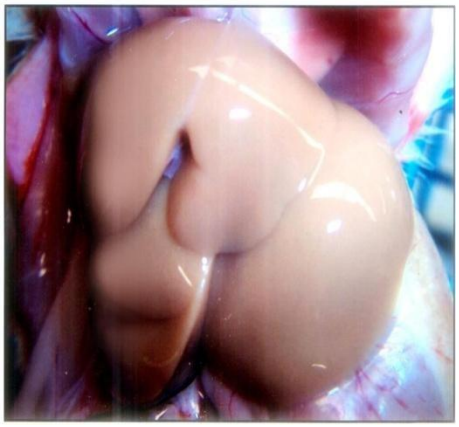

Fig 2. Isolated Rat Liver

Liver diseases and toxicity of liver[9,10,11]

Liver toxicity is mainly caused by certain chemicals and organic compounds having certain structures. Haloalkanes, certain antibiotics, chemotherapeutics, CCl4 peroxidised oil, chlorinated hydrocarbons, aflatoxin etc., excess consumption of alcohol, infections and autoimmune disorders are a few among the reasons for liver damage. The liver is unique in its function processing the chemicals and drugs, which enters the blood stream. Many of these chemicals are difficult for the kidney to excrete out of the body. The liver helps by removing these chemical from the blood stream and changing them into products that can be readily removed through bile or urine. In this processes, unstable toxic products are sometimes produced and cause injury to liver.

Acute Liver Injury: Acute liver failure develops in a short period as a consequence of viral hepatitis, drug-induced (e.g. paracetamol overdose), chemical-induced (e.g. carbon- tetrachloride) or alcohol-induced toxicity, or due to rejection after liver transplantation.

Chronic Liver Injury: Chronic cholestasis or long-term exposure to alcohol, drugs or chemicals can result in liver failure progressing into liver fibrosis. Liver fibrosis is characterized by deposition of scar tissue and its end-stage is called liver cirrhosis. Other causes of chronic liver failure are viral hepatitis, metabolic diseases like Wilson disease (copper storage disease) or auto-immune diseases (e.g. primary biliary cirrhosis, primary sclerosing cholangitis). During chronic liver injury, the endothelial cells, hepatocytes and cholangiocytes can be damaged due to the accumulation of toxic metabolites, reactive oxygen species and bile acids. This results in the activation of Kupffer cells and the recruitment of inflammatory cells and the subsequent release of growth factors (e.g. Transforming Growth Factor-β (TGF-β), cytokines, reactive oxygen species that induce the activation and proliferation of hepatic stellate cells. These cells are major players in the development of liver fibrosis. A major consequence is a remodelling of the ECM that leads to deposition of scar tissue (fibrosis) and liver dysfunction16. Therefore, the activation and proliferation of stellate cells are considered key events in liver fibrosis.

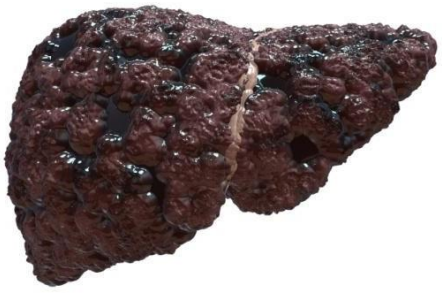

Fig 3. Chronic Liver Disease

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HEPATOTOXIC AGENTS[17-19]

These generally promote the healing process of the liver.

In India, The use of herbal products for the management of disease has a long history, starting with Ayurvedic management, and proceeding to the European and Chinese alternative systems of ancient medicines. Medicinal plants are significant sources of hepatoprotective drugs. According to one estimate, more than 700 mono- and polyherbal preparations in the form of decoction, tincture, and tablets have been used in various liver disorders. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strengths of the traditional system of medicine with that of the modern concept of evidence-based therapeutical screening, authentication, and randomized placebo-controlled clinical trials to support clinical efficacy. A large number of plants and formulations have been claimed to show hepatoprotective activities. Around 160 active constituents from 101 plants are claimed to have post liver protecting activity. In India, quite eighty seven plants square measure used in 33 patented propitiatory multi-ingredient plant formulations. In spite of the tremendous advances made, no important and safe hepatoprotective agents are obtainable in modern medicine Therefore, due importance has been given globally to develop primarily plant-based hepatoprotective medications that are effective against a range of liver disorders. A drug having helpful results on the liver is understood as a hepatoprotective drug. On the other hand, drugs having toxic effects on the liver are called hepatotoxic drugs. Clinical analysis has conjointly shown that herbals have real utility in the treatment of diseases. In the last 30 years, several hepatotoxins have been used commonly in d-galactosamine, carbon tetrachloride, acetaminophen, and thioacetamide, and more recently Concanavalin A (ConA) and lipopolysaccharide (LPS) has been developed. ConA and LPS do not reflect the clinical pattern of human disease, which indicates a great advantage in the study of cellular mechanisms involved in autoimmune liver disease. The galactosamine model is a highly selective hepatotoxin that causes liver damage similar to human viral hepatitis via depletion of uridine nucleotides, which subsequently diminishes the synthesis of RNA and proteins. Galactosamine intoxication in rats disrupts the membrane permeability of the plasma membrane, causing leakage of the enzymes form the cell, which leads to the elevation of serum enzymes. Hence, a significant rise in the transaminase levels could be taken as an index of liver damage. Galactosamine has great liver specificity compared to other toxic groups, such as paracetamol, acetaminophen, and carbon tetrachloride because hepatocytes have high levels of galactokinase and galactose-1-uridyltranseferase, and galactosamine does not affect other organs. Galactosamine induces hepatotoxicity with spotty hepatocytes, necrosis, and marked portal and parenchymal infiltration. Galactosaimine also induces the depletion of uridine diphosphate (UDP) by increasing the production of UDP-sugar derivates, which causes inhibition of RNA and protein synthesis, leading to cell membrane deterioration. The current study is aimed toward assembling information-supported reported works on promising phytochemical from herbal plants that are tested in hepatoxicity models. The review deals with fact-finding work done on herbals helpful in the treatment of liver ailments. The failure of the synthetic drugs in the treatment of hepatic diseases and the search for potent immunomodulatory agents are leading us to the world of herbal medicine in search of a product in nature for use in the protection and cure of dreaded liver diseases. Till date, there is only one protective natural drug; that, too, is not curative and also has its limitations in protecting the liver from viral attacks. The list of herbal hepatoprotective agents has been summarized in Table 1.

Table 1:- List of herbal hepatoprotective agents

S.No	Plant Name	Category	Name of Active Constituent	Mechanism
1.	*Allium sativum*	Organosulfur compounds	Organosulfur compounds	Prevention of GSH depletion, alteration of GSH-dependent enzymes
2.	*Buddleja officinalis*	Phenyl ethanoid	Acetoside	Decreased levels of AST, ALP
3.	*Camellia sinensis*	Polyphenols	Catechin	Inhibited hepatocellular apoptosis
4.	*Cistus laurifolius*	Flavanoid	Quercetin	MDA, AST level decreased.
5.	*Eglets viscose*	Flavanoid	Ternatin	Decreased lipid peroxidation
6.	*Gingko biloba*	Polyphenols	Polyprenols	ALT, AST, ALP level decreased
7.	*Hibiscus sabdariffa*	Polyphenols	Protocatechuic acid	LDH,AST, ALP Level decreased
8.	*Magnolia indica*	Triterpine	Lupeol	Decreased Level of SGOT,SGPT
9.	*Nigella sativa*	Quinones	Thymoquinonone	Scavenger of superoxide, hydroxyradical, and singlet molecular oxygen.
10.	*Ocimum basilicum*	Phenolic Acid	Rosmarinic acid	AST, ALP, SGOT level decreased.

MATERIALS AND METHODS

Plant material collection

The leaves of *Alangium salvifolium* collected from local areas of Mandidip in the month of April, and it was authenticated by Dr. Harish Kumar, Department of Botany, Ch. Sughar Singh Educational Academy, Etawah, Uttar Pradesh, India. *Evolvulus alsinoides* whole plant and *Gardenia gummifera* whole plant were collected from the fields of Thirumala hills. They were authenticated by Dr. Harish Kumar, Department of Botany, Ch. Sughar Singh Educational Academy, Etawah, Uttar Pradesh, India.

PREPARATION OF EXTRACTS

The whole plant of *Gardenia gummifera* (1 kg), *Evolvulus alsinoides* (1 kg) and leaves of *Alangium salvifolium* (2 kg) were made free from the adherent foreign material, air-dried, cut in to small pieces and coarsely powdered mechanically. Then powder of *Evolvulus alsinoides and Alangium salvifolium* were extract with ethanol, Powder of *Gardenia gummifera* was extract with methanol by using soxhlet apparatus to prepare extractions separately then solvent was removed, typically by using rotavapour. The obtained extracts were kept in a desiccator to remove moisture and stored properly until used. The concentrated product ethanolic extract of *Alangium salvifolium* coded as ASEE, ethanolic extract of *Evolvulus alsinoides* coded as EAEE and methanolic extract of Gardenia gummifera coded as GGME. A pilot study was conducted on ASEE, EAEE and GGME to fractionate it with toluene, ethyl acetate, butanone and *n*-butyl alcohol in succession separately. On ASEE studies revealed that ethyl acetate and toluene have similar TLC profile. Hence, ethyl acetate, butanone and *n*-butyl alcohol were selected for fractionation of ASEE. Toluene, butanone and *n*-butyl alcohol were selected for fractionation of EAEE. Ethyl acetate, butanone and *n*-butyl alcohol were selected for fractionation of GGME.

The extracts of ASEE, EAEE and GGME were dispersed in 1 L of distilled water separately and fractionated with toluene, ethyl acetate, butanone and *n*-butyl alcohol in succession. The obtained fractions were concentrated under reduced pressure to yield corresponding extracts.

CHEMICALS AND EQUIPMENTS

Silymarin was used as a standard hepatoprotective agent was obtained as a kind gift from Micro Labs., Ltd., Hosur (Bangalore), India; Methanol, Paracetamol, Carbon tetra chloride, methanol, DPPH, Carboxy methyl cellulose (0.5%), Tween-80, S.D Fine Chemicals, Mumbai; Olive oil-Seven Ships, Hyderabad and Normal Phase Precoated Chromatographic silica gel 60 F254 plates Merck, Germany. Serum Glutamic Oxaloacetic Transaminase(SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline phosphate (ALP), Total Bilirubin (TB), Total protein (TP), Cholesterol(CHOL) and Albumin (ALB) were purchased from Span, Diagnostics Ltd., Surat, India. All other chemicals and solvents used were of analytical grade.

UV-Visible Spectrophotometer -Elico SL149, Colorimeter-Merck Specialties Pvt. Ltd, Mumbai., Rotary vacuum evaporator(rotavapour) - Buchi, and Homogenizer, Centrifuge (R8-C Laboratory centrifuge)-Remi. Graph Pad Prism (Version 5.0) used for ANOVA.

ANIMALS

Healthy Wistar albino rats (150-200 gm) and albino mices (25-30 gm) were procured from the Advance Institute of Biotech and Paramedical Sciences. (Reg. No. 177/1999 CPCSEA), Kanpur, Uttar pradesh. Animals were housed at CPCSEA approved animal house (1533/PO/a/11/CPCSEA) with light and dark cycle and had free access to commercial pellet with water *ad libitum*. The animal house temperature was maintained at 25 ± 2 °C with relative humidity at (50 ±15%). The study were strictly followed Ethical norms during all experimental procedure.

HEPATOPROTECTIVE AND ANTIHEPATOTOXIC STUDIES

Hepatoprotective Activity [27-30]

In the present study, the animals were pretreated with test extracts/fractions before inducing liver damage with CCl4. Seven days after acclimatization, the rats were divided into nine groups (I-IX), each group consisting of six animals. All animals were kept on same diet for 7 days.

Group-I Served as a control and received 1ml/kg of vehicle in distilled water *p.o.* for seven days.

Group-II Treated with vehicle daily for seven days followed by 1 ml/kg of 50% v/v CCl4 in olive oil *p.o.* on seventh day.

Group-III (standard-Silymarin) Animals were administered with 50 mg/kg of Silymarin p.o. for 7 days followed by CCl4 administration p.o.

Group IV-IX Test groups were treated in the similar way using ASEE 150, 300 mg/kg, EAEE 75, 150 mg/kg and GGME 150, 300 mg/kg respectively followed by CCl4 administered *p.o* on the seventh day.

All the rats were anaesthetized with thiopentone sodium (60 mg/kg; *i.p.*), 36hrs after administration of CCl4, blood was collected from retro orbital by carefully opening eye of the rats. After blood collection, the blood samples were allowed to coagulate at room temperature for at least one hour. Serum was separated by centrifugation at 3000 rpm for 30 minutes and then analyzed for SGPT, SGOT, TB, ALP, TP, CHOL and ALB levels. The animals were then dissected, the livers were carefully removed, washed with 0.9% saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies.

ANTI-HEPATOTOXICITY ACTIVITY[31-33]

The animals were divided into nine groups (I-IX) each consisting of six animals. All the animals were kept on same diet for 7 days. The animals were first treated with CCl4 for inducing liver damage and then treated with extracts under investigation.

Group-I served as a control and received 1ml/kg of vehicle in distilled water *p.o.* on 1, 3 and 5th day.

Group-II served as toxic and was given 1 ml/kg of 50% v/v CCl4 in olive oil *p.o.* on 1, 3 and 5th day.

Group-III (standard-Silymarin) animals were administered with CCl4 *p.o.* on 1, 3 and 5th day, from 6th to10th i.e. for 5 days 50 mg/kg of Silymarin *p.o.*

Groups IV to IX test groups were treated in the similar way using ASEE 150, 300 mg/kg, EAEE 75, 150 mg/kg and GGME 150, 300 mg/kg respectively. Blood samples and liver of the animals were collected and processed in the similar way as mentioned in hepatoprotective activity experiment.

DETERMINATION OF SLEEPING TIME34-35.

Pentobarbitone-induced sleeping time was carried out in Swiss albino mice. A 50% v/v CCl4 in olive oil at a dose of 1.5 ml/kg/*p.o.* was used as the toxic substance for inducing liver damage.

Group-I served as a control and received 1 ml/kg of 2% gum acacia in distilled water *p.o.* for 7 days**. Group-II** served as a toxic control and were given 2% gum acacia for 7 days followed by 1.5 ml/kg of CCl4 (50% v/v CCl4 in olive oil) only on the 7th day *p.o.*

The **Group-III** (Standard) animals were administered with 50 mg/kg of Silymarin *p.o.* for 7 days followed by 1.5 ml/kg of CCl4 (50% v/v CCl4 in olive oil) only on the 7th day *p.o.*

Groups IV, V & VI were treated in the similar way to that of Group III (Standard) using ASEE 300 mg/kg, EAEE 150 mg/kg and GGME 300 mg/kg respectively.

All the various groups of animals were given Pentobarbitone 60 mg/kg; *i.p.* 2 hrs after administration of CCl4. The time between loss of righting reflex and its recovery was recorded.

STUDY OF BIOACTIVE FRACTIONS[36]

The active fractions (EAF-ASEEE, BNF-ASEE, BLF-ASEEE, TLF- EAEE, BNF-EAEE, BLF-EAEE, EAF-GGME, BNF-GGME and BLF-GGME) obtained from extracts of three plants were screened for hepatoprotective and antihepatotoxicity against carbon tetrachloride, paracetamol and ethanol induced acute and chronic hepatotoxic studies in rats as well as i*n-vitro* antioxidant activities.

EVALUATION OF PROTECTIVE EFFECT OF SELECTED ACTIVE FRACTIONS AGAINST CCL4 INDUCED LIVER DAMAGE[37-40].

The animals were divided into eight groups, each consisting of six animals. All the animals were kept on same diet for 7 days. The animals were first treated with CCl4 for inducing liver damage and then treated with extracts under investigation.

Control group received 1ml/kg of vehicle in distilled water *p.o.* on 1, 3 and 5th day.

Toxic group received 1 ml/kg of 50% v/v CCl4 in olive oil *p.o.* on 1, 3 and 5th day.

Standard group (Silymarin) Animals were administered with CCl4 *p.o.* on 1, 3 and 5th day, from 6th to10th i.e. for 5 days administered50mg/kg of Silymarin *p.o.*

Test group received 50 and 100 mg/kg; b.wt. *p.o*. of the selected fractions i.e. BLF-ASEE, BNF-EAEE, BLF-EAEE, BNF-GGME and BLF-GGME from 6th to 10th i.e. for 5 days.Blood samples and liver of the animals were collected and processed in the similar way as mentioned in hepatoprotective activity experiment.

EVALUATION OF PROTECTIVE EFFECT OF SELECTED ACTIVE FRACTIONS AGAINST PARACETAMOL INDUCED LIVER DAMAGE[41-45].

The protective effect of selected fractions against paracetamol- induced liver damage was carried out in healthy albino wistar rats. The animals maintained under standard conditions and were divided into eight groups. The animals in various groups except those in toxic group, were first treated with vehicle/Silymarin/test fractions orally for 7 days and on the 8th day, an acute oral dose of paracetamol (3 g/kg; b.wt.) in 1% w/v gum acacia was given for inducing liver damage. Silymarin and plant fractions were dispersed in 2% w/v of gum acacia in water, whereas suspension of paracetamol was prepared in 1% w/v of the same suspending agent.

Control group received the vehicle alone (2% w/v gum acacia 1 ml/kg; b.wt. *p.o.*) for 8 days.

Toxic group received the vehicle for 7 days followed by paracetamol (3 g/kg; b.wt.) in 1% gum acacia on the 8th day alone.

Standard group received with 50 mg/kg of Silymarin *p.o*. for 7 days followed by paracetamol (3.0 g/kg; b.wt.) in 1% gum acacia on the 8th day.

Test groups received 50 and 100 mg/kg; b.wt. *p.o.* of the selected fractions i.e. BLF-ASEE, BNF-EAEE, BLF-EAEE, BNF-GGME and BLF-GGME by oral route for 7 days, followed by paracetamol (3.0 g/kg; b.wt.) in 1 % w/v gum acacia on the 8th day. The total duration of the study was 8 days and the administration of fractions, standard, vehicle or paracetamol was only once on the days specified. The blood was withdrawn 24 hrs after the administration of paracetamol. The withdrawal of blood, separation of serum, dissection of liver and calculation of percentage protection of the biochemical parameters were carried out as mentioned under the hepatoprotective activity.

EVALUATION OF ANTIHEPATOTOXIC ACTIVITY OF THE POLYHERBAL EXTRACT (PHE) AGAINST CCL4 INDUCED HEPATOTOXICITY[46-48]

A polyherbal extract was prepared by selecting three bioactive fractions of different plants under investigation. The amount of the fractions depends on the efficacy exhibited on the study of the individual fraction against various hepatotoxic agents. The composition of the polyherbal extract is given in the Table 2.

The antihepatotoxic activity of PHE at a dose of 100 mg/kg; b.wt.*p.o*. was evaluated by inducing acute liver injury in rats using CCl4 hepatotoxicant. The biochemical parameters such as SGPT, SGOT, TB, ALP, TP, CHOL and ALB were determined by the methods described earlier. The livers isolated were processed as mentioned earlier and the changes occurred in hepatic architecture were evaluated.

Table 2.Composition of Polyherbal Extract

S. No	Bioactive fractions	Quantity (per illigram of the polyherbal formulation)
1.	BLF-ASEE	100 mg
2.	BLF-EAEE	100 mg
3.	BNF-GGME	50 mg

ESTIMATION OF BIOCHEMICAL PARAMETERS

The following are the biochemical parameters estimated to evaluate the effect of the test materials against the experimentally induced hepatotoxicity caused by different agents:

a) Serum glutamic pyruvic transaminase (SGPT)

b) Serum glutamic oxaloaetic transferase (SGOT)

c) Serum alkaline phosphatase (ALP)

d) Total serum bilirubin (TB)

e) Total Protein (TP)

f) Albumin (ALB)

g) Cholesterol (CHOL)

STATISTICAL ANALYSIS

Values are expressed in Mean± S.E.M. for six animals in each group and statistically assessed by one-way analysis of variance (ANOVA) and subjected to Dunnett's test. The $P<0.05$ was considered significant.

HISTOPATHOLOGICAL STUDIES

The liver is made up of hepatocytes and specialized cells called Kupffer cells interspersed with sinusoids. It is supplied with branches of bile duct. The normal hepatocytes have intact plasma membrane while in the event of viral infection/disease state or when drugs or chemicals affect liver cells, there will be changes in the permeability of plasma membrane and disruption of cells is caused by excessive formation of fibrotic tissue eventually leading to necrosis.

Histopathological studies could be carried out to assess the degree of damage. This is done by staining the fine section of liver isolates and examining under the microscope.

After the animals were sacrificed, livers were taken out and washed with normal saline (0.9%). Then, 2-3 pieces of approximately 6 mm3 size were cut and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections.

Processing of Liver Tissues: Liver pieces were taken out from fixing solution and dehydrated for 30 min each in 30, 50, 70, 90 and 100% alcohol, successively. To remove the alcohol from the dehydrated tissues, they were kept for 30 min each in alcohol: Xylene mixture (1:1) followed by pure xylene. The tissues were then kept in xylene, paraffin wax mixture (1:1) for 1h and then in molten paraffin wax at 62 °C, after which, they were trimmed and mounted on wooden blocks for thin sectioning.

Staining and Mounting of Liver Tissues: Ribbons of thin sections of liver tissues were placed in rows on clean glass slides previously coated with albumin-glycerin mixture and few drops of water added to let the sections float. The slides were heated on hot plate to fix liver sections on to the slides. The slides were then placed for 5 min each in xylene to remove wax, then in absolute alcohol to remove xylene from the liver sections. Hydration of liver sections was attained by keeping them in descending series of alcohol and water mixtures (90%, 70%, 50%, 30% alcohol and in pure water) for 3 min each. Hydrated sections were stained with haemotoxylin stain for one minute and washed in running tap water to remove excess stain. Liver sections were dehydrated again

by keeping in ascending of alcohol-water mixtures (30%, 50%, 70%, and 90% alcohol) for one minute. After that, the sections were kept for 5 min each in absolute alcohol and then in xylene. Finally, the stained liver.

Hepatoprotective Activity of Ethanolic extract leaves of *Alangium salvifolium*:

The results of the study are shown in Table.3

Biochemical Parameters: The administration of CCl4 by increased the levels of serum SGPT, SGOT, ALP, CHOL and TB levels, when compared with the control group. As well as those the animals receive CCl4 cause to decrease in serum TP and ALB levels which was also observed and these levels were reversed in both the ASEE treated groups. From the biochemical indices of the study, it is evident that the hepatoprotective effect of ASEE is proportionately increasing with the dose of ASEE-300 mg/kg, which showed highly significant activity (P<0.001), which is almost comparable to the group treated with Silymarin, a potent reference standard hepatoprotective drug.

Histopathological Studies: The histopathological studies (Figure. 4.2.) of the liver showed massive fatty changes, ballooning, gross centrilobular necrosis and loss of cellular boundaries in the toxic group. Both the standard (Silymarin 50 mg/kg; b.wt.*p.o.*) and ASEE (300 mg/kg; b.wt.*p.o.*) group showed reduced necrotic zones and ballooning degeneration when compared with the toxic group.

Table.3 Hepatoprotective activity of ethanolic extract leaves of *Alangium salvifolium* (pre-treatment) on different biochemical parameters in CCl4 induced liver damage in rats

GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALP (KA/dL)	TB (mg/dL)	CHOL (mg/dL)	ALB (gm %)	TP (gm %)
CONTROL	34.77±1.76	30.26± 2.50	16.58±2.51	0.54±0.09	61.09±4.29	3.86±0.25	6.46±0.61
TOXIC (CCl4)	87.39±3.51	77.98±2.71	60.28±4.23	4.81±0.24	131.60±8.01	1.95±0.13	3.69±0.32
Silymarin 50 mg/kg	66.60±3.28**	51.43±1.67***	30.08±1.93***	1.75±0.18***	91.48±4.99**	3.57±0.21***	6.24±0.42**
ASEE 150 mg/kg	75.92±4.27	60.40±4.03**	52.96±7.41	2.30±0.77**	106.00±5.23	2.59±0.31	4.79±0.18
ASEE 300 mg/kg	65.89±4.31***	53.93±5.01***	29.88±4.24**	1.91±0.43***	95.51±11.97**	3.50±0.33***	5.87±0.53**

n=6, values expressed as Mean ± SEM Significant*(P <0.05), ** (P<0.01), *** (P<0.001) compared with standard and toxic group using one-way ANOVA (Dunnett's test method)

Hepatoprotective Activity of Ethanolic Extract of *Evolvulus alsinoides*

The results of this study are presented in Table.4

Biochemical Parameters: The elevated levels of SGPT, SGOT, ALP, CHOL and TB levels, were significantly (P<0.001) reduced by the standard, EAEE 75 and EAEE 150. The test groups also exhibited a significant effect in increasing the reduced serum TP and ALB levels. The EAEE 150 showed a better hepatoprotective activity (P<0.001) than EAEE 75.

Histopathological Studies: The histopathological examination (Figure. 4.4.) of the liver of rats of standard, EAEE 75 and EAEE 150 showed a reduced fatty changes, necrosis and broad infiltration of lymphocytes produced by CCl4. The protective effect with EAEE 150 was almost comparable to the standard group.

Table. 4. Hepatoprotective activity of ethanolic extract leaves of *Evolvulus alsinoides* (pre-treatment) on different biochemical parameters in CCl4 induced liver damage in rats.

GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALP (KA/dL)	TB (mg/dL)	CHOL (mg/dL)	ALB(gm %)	TP (gm %)
CONTROL	48.60±3.61	43.25± 5.46	18.66±1.51	2.41±1.06	79.30±4.87	2.58± 0.22	8.75±0.29
TOXIC (CCl4)	131.70±12.76	152.4 ± 12.31	78.59±8.15	7.25±0.37	148.3±3.19	1.11± 0.06	2.55± 0.14
SILYMARIN 50 mg/kg	75.23±5.09***	69.75±3.39***	43.08±2.41***	2.75± 0.29***	99.74±2.39***	2.45±0.19***	7.09±0.09 ***
EAEE 75 mg/kg	107.90±12.7	107.8±10.56**	60.31±4.52*	5.09±0.05 *	132.9±3.07*	1.70±0.05 *	3.92±0.44 *
EAEE 150 mg/kg	85.20±5.57**	87.07±5.84***	50.99±3.81**	3.63±0.26 ***	108.4±3.99***	2.20±0.11***	5.95±0.40 ***

n=6, values expressed as Mean ± SEM Significant*(P <0.05), ** (P<0.01), * (P<0.001) compared with standard and toxic group using one-way ANOVA (Dunnett's test method).**

Hepatoprotective Activity of Methanolic Extract of *Gardenia gummifera*

The results of this study are presented in Table. 5

Biochemical Parameters: The elevated levels of SGPT, SGOT, ALP, CHOL and TB levels, were significantly (P<0.001) reduced by the standard. The test groups GGME 150 and 300 also exhibited a significant protective effect on all serum levels and also enhancing the reduced serum TP and ALB levels. The GGME 300 showed a better hepatoprotective activity (P<0.001) than GGME 150. The high percentage protection was observed with GGME 300 was also comparable to the reference standard drug Silymarin.

Histopathological Studies: The histopathological studies indicated that the hepatic damage induced by CCl4 was remarkably reduced by the standard Silymarin, GGME 150 and 300 showed a reduced fatty changes, necrosis and broad infiltration of lymphocytes produced by CCl4. The effect with GGME 300 was almost comparable to the standard group. The degree of the effect was as follows: Standard > GGME 300 > GGME 150.

Table.5. Hepatoprotective activity of methanolic extract leaves of *Gardenia gummifera* (pre-treatment) on different biochemical parameters in CCl4 induced liver damage in rats

GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALP (KA/dL)	TB (mg/dL)	CHOL (mg/dL)	ALB (gm %)	TP (gm %)
CONTROL	49.41±2.91	39.43±3.17	20.12±2.76	0.99±0.28	86.74±3.86	3.51±0.36	8.21±0.36
TOXIC (CCl4)	154.4±6.58	149.7±8.21	86.86±5.92	6.65±0.52	151.2±2.77	1.42±0.28	2.96±0.29
SILYMARIN 50 mg/kg	87.33±6.57***	83.2±7.74***	45.37±3.78***	2.86±0.44***	104±2.88***	2.96±0.06**	7.22±0.18***
GGME 150 mg/kg	123±10.55*	113±12.81*	66.75±6.47*	5.05±0.08*	121.3±9.27**	1.8±0.09	4.73±0.39*
GGME 300 mg/kg	96.45±4.33***	80.15±1.26***	53.72±2.27**	3.31±0.15***	102.7±2.49***	2.55±0.28*	6.44±0.52***

n=6, values expressed as Mean ± SEM Significant*(P <0.05), ** (P<0.01), *** (P<0.001) compared with standard and toxic group using one-way ANOVA (Dunnett's test method)

CONCLUSION

The three Indian medicinal plants viz., *Alangium salvifolium* (Family: Alangiaceae), *Evolvulus alsinoides* (Family: Convulvulaceae) and *Gardenia gummifera* (Family: Rubiaceae) are selected for the present investigation.

The *Alangium salvifolium* traditionally different parts of this plant were used for a wide range of diseases like paralysis, diarrhea, constipation, piles, vomiting, jaundice, leprosy, inflammation, hemorrhage, blood pressure and acute case of rheumatism. It is used

in Ayurveda for rabies and antidote for poisonous bites(snake bites)30. The *Evolvulus alsinoides* is used in the treatment of nervous debility, mental disturbances, depression, dysentery, strengthen, brain and memory, liver diseases (jaundice), syphilis, diarrhea, malarial fever, chronic bronchitis, asthma, dysentery, treatment of venereal diseases84, spermopiotic, cough and cold. Oil promotes the growth of hair. The resin of *Gardenia gummifera* is claimed to have number of medicinal properties such as carminative, antispasmodic, stimulant, diaphoretic, anthelmintic, antiseptic and expectorant, cardiotonic and useful in neuropathy. Administered to children in nervous disorders and diarrhea, colic, bronchitis, cough, constipation, wounds, leprosy, intermittent fever, skin diseases, cardiac debility, splenomegaly and obesity.

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