Chapter 3

Review of Literature

Always listen to the experts. They’ll tell you what can’t be done and why. Then do it.

- Robert Heinlein
3 REVIEW OF LITERATURE

3.1 HEPATOTOXICITY

Liver is an organ of paramount importance, which plays a vital role in regulating various physiological processes in the body, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principles. The damage of the liver caused by hepatotoxic agents is of grave consequences.

A hepatotoxin might be defined as any chemical agent that can produce injury to the liver. A large number and variety of compounds have been identified as hepatotoxins of chemical or experimental relevance. They may be as simple as inorganic elements and compounds or as complex as heterocyclics, steroids or peptides. The hepatic damage may involve mainly the hepatic parenchymal cells, cells of the excretory tree or both. In the case of some hepatotoxic agents, vascular structures are the primary focus of injury, acute hepatic injury may be translated into chronic liver diseases, expressed as cirrhosis or even as carcinoma.

The large volume of literature on experimental hepatotoxicity includes many facts relevant to clinical medicine. The studies conducted have been useful in understanding of the character and mechanism of hepatotoxic states in human (e.g., acute hepatic injury induced by carbon tetrachloride). Studies of experimental hepatotoxicity also provide increased understanding of chronic diseases that might be due to hepatotoxins. Experimental hepatotoxic states also have provided as useful models for study of the genesis of the tissue injury in general and the histogenesis, biochemical features and manifestations of spontaneous hepatic diseases (e.g., viral hepatitis). In particular, hepatic necrosis induced by the chemical agents has been...
useful model for the study of biochemical pathology of the death of cells.

Experimental hepatic injury is a fundamental tool for the development of diagnostic methods, and it has become traditional for tests of liver function and serum enzyme test to be evaluated in animals with experimental hepatic injury before assessing their usefulness in clinical basis.\(^6^9\).

### 3.1.1 CLASSIFICATION OF HEPATOTOXINS

Clinical and experimental observations have led to the general agreement that there are two main categories of agents that can produce hepatic injury.\(^7^0,^7^1\).

**A. Direct or predictable:**

When the drug or one of its metabolites is either directly toxic to the liver or it lowers the host immune defence mechanism. The hepatic damage produced by direct hepatotoxins may be mainly cytotoxic effect \(i.e\). hepatic necrosis, steatosis or both. The adverse effects occur in most individuals who consumed them and their hepatotoxicity is dose dependent \(e.g\). CCl\(_4\).

**B. Indirect or unpredictable or idiosyncratic:**

When the drug or one of metabolites acts as hapten and induces hypersensitivity in the host. The hepatic damage produced by indirect hepatotoxins may be mainly cytotoxic expressed as steatosis or necrosis or may be mainly cholestatic expressed as arrested bile flow with or without bile duct injury. The hepatotoxicity by this group does not occur regularly in all individuals and the effects are usually not dose related \(e.g\). acetaminophen.
3.1.2 MECHANISM OF HEPATOTOXICITY

The normal hepatocyte shown in the center of the below figure (Fig. 6) may be affected in at least six ways, labeled A to F:

A. Disruption of intracellular calcium homeostasis leads to the disassembly of actin fibrils at the surface of the hepatocyte, resulting in blebbing of the cell membrane, rupture and cell-lysis.

B. In cholestatic diseases, disruption of actin filaments may occur next to the Canaliculus, the specialized portion of the cell responsible for bile excretion. Loss of villous processes and the interruption of transport pumps such as multidrug-resistance-associated protein 3 (MRP3) prevent the excretion of bilirubin and other organic compounds.

C. Many hepatocellular reactions involve the heme-containing cytochrome P-450 system. Generating high-energy reactions that can lead to the covalent binding of drug to enzyme, thus creating new, nonfunctioning adducts.

D. These enzyme–drug adducts migrate to the cell surface in vesicles to serve as target immunogens for cytolytic attack by T cells, stimulating a multifaceted immune response involving both cytolytic T cells and cytokines.

E. Activation of apoptotic pathways by tumor necrosis factor α (TNF α) receptor or FAS may trigger the cascade of intercellular caspases, which results in programmed cell death with loss of nuclear chromatin.

F. Certain drugs inhibit mitochondrial function by a dual effect on both β–oxidation affecting energy production by inhibition of the synthesis of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, resulting in decreased ATP production and the respiratory-chain enzymes. Free fatty acids can’t be metabolized and the lack of aerobic respiration results in the accumulation of...
lactate and reactive oxygen species (ROS). The presence of reactive oxygen species may further disrupt mitochondrial DNA. This pattern of injury is characteristic of a variety of agents (Table 2), including nucleoside reverse-transcriptase inhibitors, which bind directly to mitochondrial DNA, as well as valproic acid, tetracycline and aspirin.59

Table 2: Hepatotoxins affecting various organelles

<table>
<thead>
<tr>
<th>Organelles affecting</th>
<th>Chemicals</th>
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<tbody>
<tr>
<td>Endoplasmic reticulum</td>
<td>Allyl formate</td>
</tr>
<tr>
<td></td>
<td>Carbon tetrachloride</td>
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<tr>
<td></td>
<td>Dimethylaminoazobenzene</td>
</tr>
<tr>
<td></td>
<td>Dimethylnitrosamine</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Allyl formate</td>
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<tr>
<td></td>
<td>Carbon tetrachloride</td>
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<tr>
<td></td>
<td>Dimethylnitrosamine</td>
</tr>
<tr>
<td></td>
<td>Ethionine, Hydrazine</td>
</tr>
<tr>
<td></td>
<td>Phosphorus</td>
</tr>
<tr>
<td></td>
<td>Pyrrolizidine alkaloids</td>
</tr>
<tr>
<td></td>
<td>Tannic acid, Thioacetamide</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>Beryllium</td>
</tr>
<tr>
<td></td>
<td>Carbon tetrachloride</td>
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<tr>
<td></td>
<td>Pyrrolizidine alkaloid</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Dimethylnitrosamine</td>
</tr>
<tr>
<td></td>
<td>Hydrazine</td>
</tr>
<tr>
<td></td>
<td>Pyrrolizidine alkaloid</td>
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</table>
Fig. 6: Mechanism of hepatotoxicity
Liver injury is generally indicated by elevations in serum aminotransferase levels, tests reflecting liver injury alone do not necessarily predict or indicate serious hepatotoxicity. Vague symptoms such as fatigue, anorexia, nausea, discomfort in the right upper quadrant and dark urine may be the first clues that hepatotoxicity has occurred. Drug related hepatotoxicity should be considered when such symptoms occur in conjunction with biochemical evidence of liver injury and especially with concurrent impaired liver function. The regulation of serum enzyme activity is not a function of the liver, which is more accurately assessed according to the levels of total bilirubin or conjugated bilirubin- reflecting the liver’s ability to move bilirubin from plasma into bile\textsuperscript{60,73}.

Another measurable liver function is protein synthesis, which is reflected in the albumin concentration and the prothrombin time\textsuperscript{74}. The most frequent hepatotoxic reactions evoke moderate-to-severe injury to hepatocytes with a clinical picture that resembles viral hepatitis, characterized by a rapid onset of malaise and jaundice in association with elevated aminotransferase levels. Each toxin has its own pattern of injury. If hepatocyte injury predominates, aminotransferase levels may be at least five times as high as normal. Elevations of alkaline phosphatase and bilirubin levels predominate in cholestatic syndromes. Signs of allergic reaction are absent in most patients. Acute liver failure may develop after a week or more of illness, particularly if the patient has continued the drug after the onset of symptoms\textsuperscript{75}. The disturbances of metabolism occurring in liver diseases are largely the result of failure of the parenchymal cells to carry out vital functions because of -

a) Infections or noxious agents

b) Decreased mass of functioning cells

c) Decreased blood supply
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d) Impaired nutrition
e) Reactions of other organs to liver damage

3.1.3 ROLE OF FREE RADICALS IN HEPATOTOXICITY

Free radicals generated in the body have ability to attack healthy cell and are capable of producing several diseases like liver disorder, diabetes, cancer etc\textsuperscript{76}.

Reactive oxygen species (ROS) plays a crucial role in various steps that initiate and regulate the progression of liver diseases, independently from the type of etiologic agents and ROS are involved in the liver damage induced by various toxins. The severity of ROS effects depends on individual characteristics such as age, obesity, alcohol intake, iron concentration as well as from endogenous intracellular and plasmatic antioxidant defence. The imbalance of the equilibrium between ROS production and antioxidant defence leading to a state of oxidative stress influences the transcription of several biochemical mediators (principally cytokines) able to modulate tissue and cellular events – apoptosis, fibrosis, cholestasis and regeneration which characterise the different types of liver injury\textsuperscript{77}.

There are two types of ROS- oxygen-centered radicals are superoxide anion ($\cdot$O$_2^-$), hydroxyl radical ($\cdot$OH), alkoxyl radical ($\cdot$RO) and peroxyl radical ($\cdot$ROO$^-$); oxygen centered non-radicals hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (1O$_2$). Other reactive species are nitrogen species such as nitric oxide (NO$^-$), nitric dioxide (NO$_2^-$) and peroxynitrite (OONO$^-$)\textsuperscript{78}.

Reactive oxygen species in biological systems are related to free radicals, even though there are non-radical compounds in reactive oxygen species such as singlet oxygen and hydrogen peroxide. A free radical exists with one or more unpaired electron in atomic or molecular orbital. Free radicals are generally unstable, highly
reactive and energized molecules. When free radicals steal an electron from a surrounding compound or molecule, a new free radical is formed in its place. The newly formed radical then returns to its ground state by stealing electrons from cellular structures or molecules. Thus, the chain reaction continues and can be 'thousands of events long' 79.

Clinical studies reported that ROS are associated with many age related degenerative diseases, including atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, aging pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury and periodontics.

![Diagram of ROS and their effects](image)

**Fig. 7: Cell injury due to ROS**

Free radicals or reactive oxygen species in the body can cause lipid oxidation, protein oxidation, DNA strand break and base modification and modulation of gene expression. Lipid oxidation is a free-radical chain reaction and ROS can accelerate
lipid oxidation. Cell membranes are phospholipids bilayers with extrinsic proteins are the direct target of lipid oxidation. As lipid oxidation of cell membranes increases, the polarity of lipid-phase surface charge and formation of protein oligomers increase; and molecular mobility of lipids, number of SH groups and resistance to thermo denaturation decrease. Malonaldehyde, one of the lipid oxidation products, can react with the free amino group of proteins, phospholipids and nucleic acids leading to structural modification, which induce dysfunction of immune systems. A high level of lipid oxidation products can be detected in cell degradation after cell injury or disease (liver). The increase of lipid oxidation products are found in liver disease, diabetes, atherosclerosis and inflammation \(^{80}\) (Fig. 7).

A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases. The total polyphenols play a vital role in anti-oxidization as well as in the biological functions of the plant. Other studies have also indicated that the anti-oxidative properties of polyphenols in edible plants and plant products may help prevent diseases \(^{81}\).

The flavonoids exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, anticancer activities. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidants. The presence of high phenolic and flavonoid content in the extract has contributed directly to the antioxidant activity by neutralising the free radicals \(^{82}\).
3.1.4 CLINICAL MANAGEMENT

In view of multiplicity and complexity of the liver functions, it is obvious that no single test can establish the disturbances in liver function. Thus batteries of liver function tests are employed for accurate diagnosis, to assess the severity of damage, to judge prognosis and to evaluate therapy. These tests are classified below according to their functions. The presently used agents like folic acid, multivitamins and few polyherbal preparations provide only a supportive therapy and do not play an effective role in providing hepatic protection. Hence, the search is towards finding an effective herbal hepatoprotective drug.

3.1.5 EVALUATION OF HEPATOTOXICITY

3.1.5.1 Liver function tests

The liver function tests are described below according to the major liver functions.

I] Tests for manufacture and excretion of bile

Bilirubin

a) Serum bilirubin estimation
b) Urobilinigen
c) Bromsulphalein excretion
d) Bile acids (Bile salts)

II] Serum enzyme assays

Alkaline Phosphatase (ALP)

Gamma- glutamyl transpeptidase (γ-GT)

Transaminases (Aminotransferases)

a) Aspartate transaminase (AST)
b) Alanine transaminase (ALT)
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Other serum enzymes
a) 5'-Nucleotidase
b) Lactic dehydrogenase (LDH)
c) Choline esterase

III] Tests for metabolic functions Amino acid and plasma protein metabolism
a) Serum proteins
b) Immunoglobulin
c) Clotting factors
d) Serum ammonia

IV] Immunologic tests
Nonspecific immunologic reactions
a) Smooth muscle antibody
b) Mitochondrial antibody
c) Antinuclear antibody

Antibodies to specific etiologic agents
a) Hepatitis B surface antigens (HBsAg)
b) Hepatitis B core antibody (HBc)
c) Hepatitis B e antigens (HBeAg)
d) Amoeba antibodies to Entamoeba histolytica

V] Ancillary Diagnostic tests

Ultrasonography

Precutaneous liver biopsy
3.1.5.2 Serum enzyme estimation

Intracellular enzyme such as AST and ALT are released in hepatocellular damage, thus elevating the serum level of this enzyme. Isocitrate dehydrogenase and certain isozymes of lactic dehydrogenase are also elevated in liver cell damage. Serum transaminase levels (AST & ALT) are markedly elevated in active hepatitis. As a general rule liver function test are employed either to assist in the differential diagnosis of jaundice or to detect and assess the extent of hepatocellular damage.83,84

a] Aspartate transaminase (AST)

Principle

AST was previously known as Serum glutamate oxaloacetate transaminase (SGOT). AST catalyzes the transfer of the amino group from L-aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH), then converts oxaloacetate and NADH to malate and NAD. The conversion of NADH to NAD decreases the absorbance at 340nm, the rate of which proportional to the SGOT activity.53,89

Clinical significance

Organ rich in AST are heart, liver and skeletal muscle. When any of these organs are damaged, the AST level rises in proportion to the severity of damage. In myocardial infarction AST starts increasing by 3-9 hours, peaks on second day return to normal on 4th -6th day. In hepatitis, AST peaks usually between 7-12 days and any increase up to 100 times. Increased levels AST are also found in mononucleosis, pancreatitis, trauma of skeletal muscle, renal necrosis and cerebral necrosis.53,89
b] Alanine transaminase (ALT)

Principle

ALT was previously known as Serum glutamate pyruvate transaminase (SGPT). ALT catalyzes the transfer of amino group from L-alanine to α-ketoglutarate to yield pyruvate and L-glutamate. Lactate dehydrogenase (LDH) then converts pyruvate and NADH into lactate and NAD. The conversion on NADH to NAD decreases the absorption at 340nm. The rate of decrease in absorbance is measured and is proportional to the ALT activity \(^{53,89}\).

Clinical significance

Elevation of ALT activity is found in liver and kidney diseases such as infectious or toxic hepatitis, infectious mononucleosis and cirrhosis. Moderate increase is also found in obstructive jaundice, metastasis carcinoma, hepatic congestion and myocardial infraction. The ALT levels may be decrease in patients undergoing long term hemodialysis without supplemental vitamin therapy.

c] Alkaline Phosphatase (ALP)

Principle

The substrate, p-nitrophenyl phosphate (PNPP) is hydrolysed by ALP to p-nitrophenol and phosphoric acid. Some divalent ions like Mg\(^{++}\) are added to the system which acts activators. PNPP is colourless in acid or alkaline medium while PNP is yellow in colour in the alkaline medium and colourless in the acid medium \(^{53}\).

Clinical significance

Increased ALP activity may be related to hepatobiliary bone disease. Very high ALP activity in serum is seen in patient with bone cancer and marked increased also occur in obstructive jaundice and biliary cirrhosis \(^{84}\).
**d) Gamma-Glutamyl Transpeptidase (γ-GT)**

*Principle*

The γ-GT catalyses transfer of gamma-glutamyl group from the substrate gamma-glutamyl para-nitroanilide to glycylglycine releasing free P-nitroaniline which absorbs light at 405nm. Enzyme activity is proportional to the increase absorbance at this wave length. 53, 84

*Clinical significance*

Elevated serum γ-GT levels appear to be indicative of disease of liver, biliary tract and pancrea. Serum γ-GT activity is usually elevated in the cases of cholesystitis, cholangitis, cholelithiasis, chronic hepatitis, viral hepatitis and metastatic carcinoma. γ-GT is particularly helpful in clinical assessment of alcoholic cirrhosis. Since serum γ-GT is not elevated in any form of bone disorder, it assay has been valuable in differentiating bone and liver disease in conjunction with alkaline phosphatase determination.

**e) Total Protein (TP)**

A healthy functioning of the liver is required for the synthesis of the serum proteins, except for the gamma globulins. The proteins synthesized in the liver are usually decreased in hepatocellular disease, but the immunoglobulins are increased in viral hepatitis and chronic liver infections. 84

**f) Albumin**

Albumin is decreased in chronic liver diseases and is generally accompanied by an increase in the beta and gamma globulins as a result of production of immunoglobulin-G (IgG) and immunoglobulin-M (IgM) in chronic active hepatitis and of IgM and immunoglobulin-A (IgA) in biliary or alcoholic cirrhosis, respectively. 84
g] Total Bilirubin (TB)

Bilirubin has been used to evaluate chemically induced hepatic injury. It is the principle pigment in the bile and is derived from the breakdown of hemoglobin when senescent red blood cells (RBCs) are phagocytosed. As most of the liver diseases are accompanied by jaundice, the differential diagnosis of jaundice plays an important role in elucidating hepatic dysfunction. An elevated level of serum bilirubin may be produced. It shows severe parenchymal injury\textsuperscript{84,85}.

h] Direct Bilirubin (DB)

Unconjugated bilirubin is not water-soluble. It is transported in the blood stream bound to albumin. It accounts for 30-50\% of bilirubin rise in hepatocellular disease or cholestasis. Unconjugated hyperbilirubinemia is most often due to either haemolysis or Gilbert's syndrome, an inherited abnormality of bilirubin metabolism\textsuperscript{84,85}.

i] Lactate Dehydrogenase (LDH)

The LDH is localized in the cytoplasm of the cells and this is extruded into the serum when the cells are damaged or necrotic. When only a specific organ, such as liver is known to be involved, the measurement of total LDH is useful\textsuperscript{84,85}.

j] Total Cholesterol (TC)

Serum cholesterol comprises two forms, free cholesterol and esterified cholesterol. In jaundice and parenchymatous liver disease, serum cholesterol level will fall. Drug administration will rectify the defective mechanism associated with carbon tetrachloride administration\textsuperscript{84,85}.

k] Triglycerides (TG)

Immediately after carbon tetrachloride administration, the TG level in the liver is elevated. The defect in the transport of TGs into the plasma is the cause for
accumulation of lipids in the liver during carbon tetrachloride intoxication. Within 3-5 hours after administration of carbon tetrachloride, decrease in serum TG level occurs in rats. Carbon tetrachloride intoxication evokes a defect in the secretory mechanism of TGs in the liver, resulting in accumulation of lipid in liver. A reduction in the synthesis of lipoproteins will result in the lower transport of TGs, which is associated with lipoprotein84,85.

3.1.6 PRECLINICAL SCREENING MODELS OF HEPATOTOXICITY

3.1.6.1 Toxic Chemicals-induced liver damage (in vivo models):

A toxic dose or repeated doses of a known hepatotoxin (carbon tetrachloride, paracetamol, thioacetamide, alcohol, D-galactosamine, allylalcohol, etc.) is administrated, to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. If the hepatotoxicity is prevented or reduced by the pre-treatment or after toxin challenge then it is inferred that the test substance is effective. Liver damage and recovery from damage are assessed by measuring serum marker enzymes, bilirubin, histopathological changes in the liver, biochemical changes in liver (Eg: hydroxyproline, lipid, etc.) and bile flow. When the liver is damaged, liver-enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST) and alkaline phosphatase (ALP) enter into the circulation. An increase in the levels of these marker enzymes in the serum is an indication of liver damage. Other effects of induced liver damage such as reduction of prothrombin synthesis giving an extended prothrombin time and reduction in clearance of certain substances such as bromosulphthalein can be used in the evaluation of hepatoprotective plants86,87.

The hepatoprotective effect of a drug against different hepatotoxins differs especially when the mechanism of action of toxins are different. Therefore, the
efficacy of each drug has to be tested against hepatotoxins, which act by different mechanisms.

Since the CCl₄ and paracetamol induced hepatic injury models are adapted, mechanisms of hepatic injury induced by these substances are explained below.

A) Mechanism of carbon tetrachloride induced Hepatotoxicity

Initially, carbon-halogen bond is cleaved by cytochrome P-450 to form chloride anion and trichloromethly radical (CCl₃*).

\[ \text{Cytochrome P-450} \quad \text{CCl}_4 \quad \rightarrow \quad \text{Cl}^* + \text{CCl}_3^* \]

CCl₄ is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury. CCl₄ induces fatty liver and cell necrosis and play a significant role in inducing triacylglycerol accumulation, depletion of GSH, increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzyme activity. Being cytoplasmic in location the damage marker enzymes AST, ALT and LDH are released in the serum\(^88\). It is now generally accepted that the hepatotoxicity of CCl₄ is the result of reductive dehalogenation, which is catalyzed by cytochrome P-450 enzyme and forms the highly reactive trichloromethyl free radical. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. The free radical can form covalent bond with sulfahydryl group, such as glutathione (GSH), protein thiol and lipids or abstracting a hydrogen atom from an unsaturated lipid. This covalent binding of free radical to cell macromolecules is considered the initial step in a chain of events, which eventually leads to membrane lipid peroxidation, liver damage and finally cell necrosis\(^89,90\).
CCI₄ is reductively converted by Cyt. P-450 to the trichloromethyl radical the fate of this radical is of interest. First the radical add covalently to unsaturated fatty acids, trichloromethyl fatty acids, particularly of membrane phospholipids.

Recently these substituted fatty acids have been noted to be partially resistant to replace from endoplasmic reticular phospholipase A2. This seems to be result of cross linking of trichloromethyl fatty acid radical, which adds to double bond of another adjacent fatty acids (link)⁹⁰.

The physiologic significance of this cross-linking on membrane structure and function may be of great importance, particularly if these phospholipids are transformed to other critical sites in the cell. Besides covalent binding to lipid, the cells can abstract an electron from unsaturated fatty acids, yielding CHCl₃ and or fatty acid radical. Either the trichloromethyl fatty acid radical or the fatty acid radical can react with oxygen to form peroxy radical, which initiates the lipid peroxidation chain reaction⁹¹.

Administration of a single dose of CCl₄ to a rat produces within 24 hrs a centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver within 3 hrs of administration. Thereafter, the level falls and by 24 hrs there is no CCl₄ left in the liver. The development of necrosis is associated with leakage of hepatic enzymes into serum⁹².

B) Mechanism of Paracetamol induced Hepatotoxicity:

Paracetamol (PCM) is a widely used analgesic and antipyretic drug and is safe when used in therapeutic doses. However, over dosage of paracetamol is known to be hepatotoxic and nephrotoxic in man and in experimental animals. Paracetamol is a direct hepatotoxin i.e. intoxication is dose dependent and reproducible⁹³. Exposure of animals to higher doses produces centrilobular or massive hepatic necrosis followed
by congestion and failure. The hepatic necrosis is associated with damage to subcellular organelle including mitochondria. Thus the drug is used as a typical hepatotoxin to produce hepatic failure experimentally. It is established that, a fraction of PCM is converted via the cytochrome P-450 (CYP-450) pathway to a highly toxic metabolite; N-acetyl-p-benzoquinamine (NAPQI) which is normally conjugated with glutathione and excreted in urine. Overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction and the development of acute hepatic necrosis. Several cytochrome P-450 enzymes are known to play an important role in PCM bioactivation to NAPQI. The CYP-450 2E1 has been suggested to be primary enzyme for PCM bioactivation in liver microsomes. Studies demonstrated that PCM induced hepatotoxicity can be modulated by substances that influence P-450 activity. In the assessment of liver damage by PCM the determination of enzyme levels such as AST, ALT is largely used. The necrosis or membrane damage releases the enzyme into circulation and hence, it can be measured in the serum\textsuperscript{94,95}.

3.1.7 LIVER DISEASES AND INDIGENOUS MEDICINAL PLANTS

In spite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. In the absence of reliable liver-protective drugs in modern medicine, there are a number of medicinal preparations in traditional systems of medicine recommended for the treatment of liver disorders and their usage is in vogue since centuries and are quite often claimed to offer significant relief\textsuperscript{96}. The wide and ancient use of some plants to treat many liver pathologies by Oriental and Folk medicine, lead several researchers to study antioxidants, both synthetic and natural,
Table 3: Plants having liver protective property against toxic chemical induced liver damage in experimental animals

<table>
<thead>
<tr>
<th>1. Acacia catechu</th>
<th>16. Phyllanthus emblica</th>
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<tbody>
<tr>
<td>2. Achillea millefolium</td>
<td>17. Phyllanthus debilis</td>
</tr>
<tr>
<td>3. Azadirachta indica</td>
<td>18. Phyllanthus kozhikodianus</td>
</tr>
<tr>
<td>5. Boerhaavia diffusa</td>
<td>20. Phyllanthus niruri</td>
</tr>
<tr>
<td>7. Chelidonium majus</td>
<td>22. Ricinus communis</td>
</tr>
<tr>
<td>8. Cichorium intybus</td>
<td>23. Sida cordifolia</td>
</tr>
<tr>
<td>10. Eclipta alba</td>
<td>25. Swertia chirata</td>
</tr>
<tr>
<td>12. Glycosmis pentaphylla</td>
<td>27. Tinospora cordifolia</td>
</tr>
<tr>
<td>14. Moringa oleifera</td>
<td>29. Wedelia calendulacea</td>
</tr>
<tr>
<td>15. Ocimum sanctum</td>
<td>30. Withania somnifera</td>
</tr>
</tbody>
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Table 4: Some of the polyherbal formulations verified for their anti-hepatotoxicity against toxic chemical-induced liver damage in experimental animals

<table>
<thead>
<tr>
<th>Liv.52</th>
<th>Livol</th>
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<tbody>
<tr>
<td>Liv.42</td>
<td>B.Liv.</td>
</tr>
<tr>
<td>Jigrine</td>
<td>Hepatomed</td>
</tr>
<tr>
<td>Liver cure</td>
<td>Tefroli</td>
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<tr>
<td>Icterine</td>
<td>Livex</td>
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for their ability to prevent liver damage and modulate injury progression\textsuperscript{97}. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations\textsuperscript{98}. Only a small portion of the hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficacy. Several plants were reported as hepatoprotective against hepatotoxicity in animals by Indian investigators during last decade\textsuperscript{98} (Table 3). Some of the polyherbal formulations are verified for their hepatoprotective action against chemical induced liver damage in experimental animals\textsuperscript{99} (Table 4).

Livex, a compound herbal formulation from Ban laboratories (Rajkot), was investigated for its possible hepatoprotective effect in Wister rats against erythromycin estolate induced toxicity. Oral administration of Livex significantly prevented the occurrence of erythromycin estolate induced hepatic damage. The increased level of serum enzymes (aspartate transaminase, alanine transaminase, alkaline phosphatase), bilirubin, serum and tissue cholesterol, triglycerides, phospholipids and free fatty acids observed in rats treated with erythromycin estolate were very much reduced in rats treated with Livex. Results of this study revealed that Livex could afford a significant protection against erythromycin estolate induced hepatocellular damage\textsuperscript{100}.

The Polyherbal suspensions were formulated using extracts showing significant activity and evaluated for both physicochemical and hepatoprotective activity in comparison with Liv. 52 as standard. Petroleum ether, chloroform, benzene, ethanol and aqueous extracts of \textit{Ferula asafetida}, \textit{Momordica charantia} Linn and \textit{Nardostachys jatamansi} were evaluated for hepatoprotective activity against carbon tetrachloride-induced liver toxicity in Wistar rats. Polyherbal suspensions were prepared by the trituration method using a suspending agent and other excipients.
Formulation F3 has shown significant hepatoprotective effect by reducing the elevated serum enzyme levels such as glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and alkaline phosphatase. These biochemical observations were supplemented by histopathological examination of liver sections. Various parameters evaluated for all formulations were within the official specifications. Experimental data suggested that treatment with formulation F3 enhances the recovery from carbon tetrachloride-induced hepatotoxicity. From these results it may be concluded that the F3 formulation (containing chloroform, petroleum ether and aqueous extracts of Ferula asafetida, petroleum ether and ethanol extracts of Momordica charantia Linn. and petroleum ether and ethanol extracts of Nardostachys jatamansi) demonstrated significant hepatoprotective activity that might be due to combined effect of all these extracts.

Hepatoprotective activity of the polyherbal hepatoprotective formulation (PHF) containing spray-dried aqueous extracts of Andrographis paniculata Nees, Phyllanthus niruri Linn. and Phyllanthus emblica Linn. was screened against paracetamol, carbon tetrachloride (CCl₄), and ethanol-induced hepatic damage in rats. PHF was evaluated by measuring levels of serum marker enzymes like SGOT, SGPT, ALP, direct bilirubin (DB), and lactate dehydrogenase (LDH). The histological studies were also studied support the biochemical parameters. Results suggest that the hepatoprotective effects of PHF might be useful for liver protection due to combined action of all plant extracts along with their phytoconstituents.

The effect of Himoliv (HV) was evaluated in carbon tetrachloride or paracetamol induced hepatotoxicity in rats. Liver necrosis was produced by administering single dose of either carbon tetrachloride (CCl₄, 1 ml/kg, 50% v/v with olive oil, s.c.) or paracetamol (PCM, 1 g/kg, p.o.). The liver damage was evidenced by
elevated levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (ALP) and hepatic thiobarbituric acid reacting substances (TBARS) and superoxide dismutase (SOD). HV pretreatment (0.5 and 1.0 ml/kg, p.o.) significantly (P<0.001) reduced CCl₄ or PC-induced elevations of the levels of SGOT, SGPT, ALP and TBARS, while the reduced concentration of SOD due to CCl₄ or PC was reversed.

The combined hepatoprotective effect of Bi-herbal ethanolic extract (BHEE) was evaluated against carbon tetra chloride (CCl₄) induced hepatic damage in rats. Ethanolic extract from the leaves of *Eclipta alba* and seeds of *Piper longum* at a dose level of 50 mg/kg body weight was administered orally daily once for 14 days. The results of this study strongly indicate that formulation has got a potent hepatoprotective action against CCl₄ induced hepatic damage in rats.

A study was carried out in 20 consecutive patients who suffering from liver disorders, a herbal formulation (*Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentals, Achillea millefolium, Tamarix gallica*) has been clinically evaluated for its efficacy and safety in liver disorders at a dose of 1 capsule twice a day for 30 days. Clinical symptoms and biochemical parameters were evaluated on entry and at the end of study period. The results indicate that the herbal formulation produces significant improvement in clinical and biochemical parameters when given for a period of 30 days. The drug was well tolerated and did not produce any adverse effects. This herbal formulation appears to be a safe and effective medication in liver disorders. In this study it was found that the herbal formulation possesses significant hepatoprotective activity in various liver disorders and the formulation is safe without any serious adverse effect. 
Conducted a study for demonstrated the efficacy of six commercially available polyherbal hepatoprotective liquid formulations, namely Liv. 52, Livergen, Livokin, Octogen, Stimuliv and Tefroliv in acute liver toxicity in mice model induced by paracetamol (PCM). The pretreatment in low doses (2.6 ml/kg/day) with liquid formulations of Liv. 52 and Livergen reversed the PCM induced liver toxicity. At higher doses (5.2 ml/kg/day), all the six herbal formulations conclusively showed marked beneficial effects in the studied pharmacological, biochemical and histological parameters. However, it suggests that a dose adjustment may be necessary to optimize the effects in clinical settings106.

Livobond, a polyherbal formulation from Unjha Pharmacy was evaluated for its hepatoprotective effect against CCl₄-induced hepatocellular injury in rats. The toxic effects of CCl₄ in Livobond treated group was controlled significantly by restoration of the levels of serum bilirubin, proteins and enzymes as compared to the CCl₄ treated and silymarin treated groups. The results suggest that Livobond is able to significantly alleviate the hepatotoxicity induced by CCl₄ and may be attributed to the antioxidant property of the formulation107.

There is greater need for the development of formulation to combat the liver disease caused by the routinely using therapeutics as well accidentally met hepatotoxins. In this research project, we have formulated a hepatoprotective polyherbal formulation comprising of extracts of Coccinia indica, Sida cordata and Scoparia dulcis. The development of polyherbal formulation was systematic comprising of in-vitro as well as in-vivo studies.
3.2 **COCCINIA INDICA WT. AND ARN.**

**Family:** Cucurbitaceae

**Synonyms:** *Coccinia grandis* Linn, *Cephalandra indica* Naud

**Vernacular Names:**
- Hindi: Kundru
- English: Ivy-Gourd
- Marathi: Tondli, Tindora
- Sanskrit: Bimbi, Tundi
- Tamil: Kovakka
- Gujarati: Kadavighilodi, Ghilodi

**Scientific Classification:**

- **Kingdom:** Plantae – Plants
- **Subkingdom:** Tracheobionta – Vascular plants
- **Super-division:** Spermatophyta – Seed plants
- **Division:** Magnoliophyta – Flowering plants
- **Class:** Magnoliopsida – Dicotyledons
- **Subclass:** Dilleniidae
- **Order:** Cucurbitales
- **Family:** Cucurbitaceae - Cucumber family
- **Genus:** *Coccinia* Weight & Arn.
- **Species:** *Coccinia indica* Weight & Arn.
Description:

*Coccinia indica* Wight and Am (Fam. Cucurbitaceae) a climbing or prostrate, much branched, perennial herb with tuberous rootstock producing annual stem up to several meters long, which is found spreading on ground and twilling around the tree or supports around it. Leaves are triangular or pentagonal in shape. Margin is denate, upper surface glabrous and attachment of petiole and major vein branching occurs. Apex obtuse, petioles 1-3 cm long and tendrils are unbranched. Flowers monoecious, solitary, rarely in axillary clusters of 2-3, pedicels 15-50 mm long, corolla lobes white, ovate, hypanthium 10-15 mm long. Fruit is slimy in touch, pulpy and ovoid to ellipsoid shaped. It is green in colour when young which turns to scarlet red when it ripe, 2.5-5 cm long and 1.3-2.5 cm in diameter, glabrous, pulp red. The fruit possesses numerous seeds which are oblong, 6-7 mm long, margins thickened. Leaves are simple, sub glossy, alternate, ovate, palmate 3-5 lobes with obtuse apex, ranging from 5-8 cm long and 3-6.5 cm wide. It shows reticulate venation with glabrous surface, dentate margin and cordate base. Petiole stout, cylindrical, smooth, 1.2-3.0 cm long, slightly glossy. The leaves have bright green upper surface and pale-green underneath, with characteristic odour and astringent taste\(^{108,109}\).

Distribution:

*Coccinia indica* is cultivated in Assam, West Bangal, Bihar, Orissa, Maharashtra, Andhra Pradesh, Tamil Nadu; wild in many parts of India\(^{109}\).

Use in Ayurveda and Unani:

In Ayurveda it is used as galactagogue, antipyretic; cures leprosy ‘vata’ the burning sensation of the body, consumptions, jaundice, diseases of blood and in inflammation. It was also given as an anthelmintic. According to Kaiyadeva Nighantu, the bitter variety was used.
In Unani medicine, the juice of Kanduri is given in polyuria; root bark as a purgative; flowers in bilious affections, liver and skin diseases; fruits as blood-purifier, astringent, for direct action on kidneys and urinary disorders\textsuperscript{109, 110}.

**Ethnobotanical Uses:**

The whole plant is traditionally used for various medicinal purposes like for diabetes, glycosuria, enlarged gland and skin diseases\textsuperscript{108-112}.

**Summary of the Traditional Uses:**

- **Fruits:** used to cures sores on tongue and eczema, also raw fruit used as vegetable.
- **Leaf:** used in treatment of diabetes, wounds, ulcer, inflammation, in eruptions of skin, fever, asthma and cough.
- **Stem:** used as asthma and bronchitis, gastro-intestinal disturbance and diseases, Urinary tract infection and related troubles,
- **Root:** used for remove pain in joints, apthous ulcers, wheezing and phlegm, cure diabetes and intermittent glycosuria, skin diseases, skin lesions.

**Phytoconstituents:**

Whole plant contains carbohydrates, glycosides, fix oils and fats, proteins and amino acids, saponins, tannins, phytosterol, alkaloids, phenolic compounds, flavonoids, gum and mucilage, aspartic acid, glutamic acid, asparagine, tyrosin, histidine. Fruits yields taraxerone, taraxerol and (24R)-24- ethylcholest- 5- en-3\textsubscript{β}- ol gluciside, \textbeta-carotene, lycopene, cryptoxanthin and \textbeta-sitosterol, \textbeta-amyrin and its acetate lupeol, steroids, saponins, ellagic acid, lignin's, triterpenoids, in addition to alkaloids, tannins, flavonoids, glycosides, phenols and cucurbitacin B. Leaves exhibited anthraquinons in addition to alkaloids, carbohydrate, proteins and amino acids, tannins, saponins, flavonoids, phytosterol, triterpenes. Aerial parts show
presence of heptacosane, cephalandrol, tritriacontane, β-sitosterol, alkaloids cephalandrines A and B, carotenoids and cryptoxanthin\textsuperscript{112-116}.

**Scientific reports:**

**Antibacterial activity**

The aqueous and organic solvent (Petroleum ether, chloroform and ethanol) extracts from the leaves of *Coccinia indica* (Cucurbitaceae) were tested against *Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus subtilis* and *Salmonella typhimurium* by agar well diffusion method and broth dilution method. Results showed promising antibacterial activity against the bacteria tested. Among these, ethanol and aqueous extracts were found to have a more potent inhibitory effect comparing with the other extracts\textsuperscript{116}.

**Hepatoprotective activity**

β-sitosterol isolated from fruits and leaves of *Coccinia indica* which has antihepatotoxic action as evidenced from restoration of serum enzyme levels and hepatic antioxidant activities towards normalcy in comparison with hepatoprotetive drug silymarin in CCl\textsubscript{4} induced hepatotoxic rats. The Ci compound (β-sitosterol) also revealed significant dose dependent reduction in the hepatic antioxidant enzyme activities such as super oxide dismutase, glutathione, catalase, and peroxidase. The structural characterization of β-sitosterol based on UV, IR and NMR spectroscopy and Mass spectrometry\textsuperscript{117}.

Hepatoprotective effect of crude ethanolic and aqueous extracts from the leaves of *Coccinia indica* against liver damage induced by CCl\textsubscript{4} in rats. The ethanolic extract at an oral dose of 200 mg kg\textsuperscript{-1} exhibited a significant (P<0.05) protective effect as shown by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin and total cholesterol and
increasing level of total protein and albumin levels as compared to silymarin, the positive control. These biochemical observations were supported by histopathological examination of liver sections. The activity may be due to presence of flavonoid compounds\textsuperscript{118}.

Aqueous, light petroleum, chloroform, alcohol, benzene and acetone extracts of the leaves of \textit{Coccinia indica} (Family: Cucurbitaceae) were screened for antihepatotoxic activity. The extracts were given after the liver was damaged with CCl\textsubscript{4}. Liver function was assessed based on liver to body weight ratio pentobarbitone sleep time, serum levels of transaminase (SGPT & SGOT), alkaline phosphatase (SALP) and bilirubin. Alcohol and light petroleum was found to have good anti hepatotoxic activity\textsuperscript{119}.

Ovicidal and repellent properties

The ovicidal activity was determined against three mosquito species to various concentrations ranging from 50-300 ppm under the laboratory conditions. The hatch rates were assessed 48 h post treatment. The repellent efficacy was determined against three mosquito species at three concentrations viz., 1.0, 2.5 and 5.0 mg/cm\textsuperscript{2} under the laboratory conditions. From the results it can be concluded the crude extract of \textit{Coccinia indica} was an excellent potential for controlling \textit{Culex quinquefasciatus}, \textit{Aedes aegypti} and \textit{Anopheles stephensi} mosquitoes and the repellent activity was dose dependent\textsuperscript{120}.

Wound healing Activity

Herbal gel containing ethanolic fruit extract and aqueous fruit extract of \textit{Coccinia indica} was formulated and evaluated on excision wound model and incision wound model. Excision wound measuring about 500 mm\textsuperscript{2} was created on the albino rats placed in group (n=6) and the gel applied topically on the wounded area which
was measured at interval of 3 days until epithelization and complete wound closure. Blank gel and Framycetin sulphate cream (FSC) 1% w/w served as the control and standard treatment respectively. Topical application of ethanolic extract gel on excision wound in rats caused a significant ($P<0.01$) higher rate of wound healing (99.49%) and reduced epithelization period. In incision wound model, ethanolic extract gel significantly ($P<0.01$) increased the breaking strength as compared to control (486.10±5.86) than aqueous extract (415.78±6.43). The result suggest that treatment with ethanolic extract gel of *C. indica* fruits may have beneficial influence on the various phases of wound healing like wound contraction and resulting in faster healing than aqueous extract\textsuperscript{121}.

**Anti-diabetic Activity**

The combined effect of *Abroma augusta* and *Coccinia indica* known to be useful for the treatment of diabetes in Ayurveda on the fasting blood sugar, glucose tolerance and lipid profile of Streptozotocin (STZ) induced albino rats. 300mg of water extract of the mixture of dried powdered roots of *A. augusta* and leaves of *Coccinia indica* in equal proportions was given once daily for 8 weeks. After 8 weeks of treatment of Streptozotocin (STZ) diabetic rats, the fasting blood sugar came down to almost normal value and improvement in glucose tolerance and serum lipid profile were also observed. Water extract of combination *A. august* plus *C. indicia* has got good hypoglycemic and hypolipidemic effect and also corrects complications associated with diabetes such as, retinopathy, nephropathy, neuropathy and musculopathy\textsuperscript{122}.

Hypoglycemic activities of leaves of *Coccinia indica* were tested with 90% alcoholic extract. Diabetes was induced by a single intraperitoneal injection of a freshly prepared of streptozotocin 55 mg/kg b.w. of rats in 0.1 M citrate buffer.
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Review of Literature

Alcoholic extract 600 mg/kg b.w. was injected orally to mice. Oral administration of alcoholic extract of leaves of Coccinia indica shows significant hypoglycemic effect on blood glucose level in normal fasted rats\textsuperscript{123}.

To evaluate the effectiveness of Coccinia cordifolia (C. indica) on blood glucose levels of incident type 2 diabetic patients requiring only dietary or lifestyle modifications. The study was a double-blind, placebo-controlled, randomized trial. Sixty incident type 2 diabetic subjects (aged 35-60 years) were recruited from St. Johns Medical College Hospital, Bangalore, India. The subjects were randomly assigned into the placebo or experimental group and were provided with 1 g alcoholic extract of the herb for 90 days. All subjects were provided with standard dietary and physical activity advice for blood sugar control. There was a significant decrease in the fasting, postprandial blood glucose and A\textsubscript{1}C of the experimental group compared with that of the placebo group. The fasting and postprandial blood glucose levels of the experimental group at day 90 significantly decreased, by 16 and 18\%, respectively. There were no significant changes observed in the serum lipid levels. This study suggests that Coccinia cordifolia extract has a potential hypoglycemic action in patients with mild diabetes\textsuperscript{124}.

The oral administration of the pectin isolated from the fruit of Coccinia indica, at a dose of 200 mg/100 g bw/day showed a significant hypoglycaemic action in normal rats. The pectin administration resulted in a significant reduction in blood glucose and an increase in the liver glycogen. Glycogen synthetase activity was highly significant. Incorporation of labelled glucose into hepatic glycogen was also found to be higher. A significant reduction in phosphorylase activity was noted in the pectin-administered groups\textsuperscript{125}. 

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Antitussive activity

Methanol extract of fruits of *Coccinia grandis* with two different concentrations (2.5% and 5% w/v) was tested for antitussive activity by counting number of cough bouts produced due to aerosol and sulfur dioxide. The extract showed significant inhibition of cough, like the standard drug (Codeine phosphate) in dose-dependent manner\(^\text{126}\).

Antihelminthic activity

Antihelminthic activity of *Coccinia indica* (fruits) using petroleum ether, ether acetate, methanol and aqueous extracts was studied. Different concentrations of extracts were used for antihelminthic activity (25 and 50 mg/mL). *Pheretima posthuma* worms were used for antihelminthic activity. Antihelminthic activity of *C. indica* was confirmed by examine the time taken for paralysis (P) and death (D) for worms in minutes. Methanolic extract of *C. indica* exhibited antihelminthic activity in dose dependent manner taking shortest time for paralysis \([P= (4.5±0.64 \text{ min})]\) and death \([D= 6.5±0.64 \text{ min}]\) with 50 mg/ml concentration\(^\text{127}\).

Antioxidant property

The study was carried out to investigate the antioxidant effect of an ethanolic extract of *Coccinia indica* leaves, an indigenous plant used in Ayurvedic Medicine in India, in Streptozotocin-diabetic rats. Oral administration of *Coccinia indica* leaf extract (CLEt) (200 mg/kg body weight) for 45 days resulted in a significant reduction in plasma thiobarbituric acid reactive substances, hydroperoxides, vitamin E and ceruloplasmin. The extract also caused a significant increase in plasma vitamin C and reduced glutathione, which clearly shows the antioxidant property of CLEt. The effect of CLEt at 200 mg/kg body weight was more effective than glibenclamide\(^\text{128-129}\).
Anti-inflammatory, analgesic and antipyretic activity

Anti-inflammatory activity of the aqueous extract of fresh leaves of *Coccinia indica* was studied in rats using the carrageenan-induced paw edema method at various dose levels. Analgesic and antipyretic properties were evaluated using tail flick model and yeast-induced hyperpyrexia, respectively. Ceiling effect of the extract was observed at 50 mg/kg in pre-treatment carrageenan test. The effect was equivalent to diclofenac (20 mg/kg) at 50 mg/kg but it was significantly pronounced at higher doses. Effectiveness of extract in the early phase of inflammation suggests the inhibition of histamine and serotonin release. The extract produced marked analgesic activity comparable to morphine at 300 mg/kg, which suggests the involvement of central mechanisms. A significant reduction in hyperpyrexia in rats was also produced by all doses of extract with maximum effect at 300 mg/kg comparable to paracetamol. In conclusion, this study has established the anti-inflammatory activity, analgesic and antipyretic activity of *C. indica* and, thus the ethnic uses of the plant were justified\(^{114}\).

Antidyslipidemic activity

Ethanol extract of *C. grandis* showed significant triglyceride and cholesterol lowering effects in dyslipidemic hamaster model. Ethanolic extract was fractionated into chloroform, n-butanol and water soluble fractions (250 mg/kg bw) which were used to evaluate the antidyslipidemic activity. Standard drug Fenofibrate at the dose of 108 mg/kg b.w. was used. Chloroform fraction was found to possess significant lipid lowering activity followed by increase in high density lipoprotein. Chloroform soluble fraction which acts as active fraction was subjected to repeated column chromatography for isolation of a polypreanol compound and characterized as C60-polyprenol\(^{130}\).
3.3 *SIDA CORDATA* (Burm.f.) Borss.

**Family:** Malvaceae.

**Synonyms:** *Sida veronicifolia* Lam.,

*Sida humilis* Cav.\(^{131}\)

**Vernacular Names:**
- Hindi: Bananiyar, Bhiunli.
- English: Heartleaf fanpetals
- Marathi: Bhojabal, Bhuichikna
- Sanskrit: Bhumibala
- Tamil: Palampasi
- Gujarati: Bhojabala

**Scientific Classification:**

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<th>Plantae – Plants</th>
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<td>Genus</td>
<td><em>Sida</em> L.- Fanpetals Species</td>
</tr>
<tr>
<td>Species</td>
<td><em>Sida cordata</em> (Burm.f.) Borss.</td>
</tr>
</tbody>
</table>
- Heartleaf fanpetals
Description:

The plant possesses a perennial much branched herb; branches prostrate or trailing, sometime rooting, more or less hairy leaves 1 to 2.5 cm long cordate ovate, acute or acuminate, serrate, sparsely clothed with stellate hairs, petioles 1 to 2.2 cm long. Pedicels 1 to 2-3.8 cm long slender, axillary, solitary or twin, jointed a little above middle. Calyx 4mm long, 5 angled, hairy, lobes triangular, acute, or acuminate. Corolla pole yellow, slightly exceeding the calyx. Carpels 5, smooth, not reticulated, muticous or with a small slight 2-lipped beak, not cuspidate. Seeds brown, glabrous\textsuperscript{131,132}.

Distribution:

\textit{Sida cordata} is a perennial medicinal herb distributed throughout hotter parts of India, Pakistan and other tropical countries\textsuperscript{133}.

Use in Ayurveda and Siddha

\textit{Sida} species found in India are known by general name Bala and referred under ‘Bala Chatustaya’ in Ayurvedic system of medicine. The drug Bala is a reputed drug in Ayurveda is used for the treatment of rheumatism and it forms a chief ingredient of several important Ayurvedic preparations like Ksirabala, Dhanvantaram, Balaristam, Rasnadhi kasayam, Asvagandhadi leham etc\textsuperscript{134}. Root is the officinal part of the drug and is reported to be cool, sweet, demulcent, aphrodisiac and tonic. It produces strength, imparts beauty to the body and cures vatarakta, raktapitta, consumption, polyuria and ulcers. The drug is also useful in neurological disorders like hemiplegia, facial paralysis and sciatica, general debility, headache, ophthalmia, dysuria, leucorrhoea, tuberculosis, diabetes, fever and uterine disorders\textsuperscript{135}. Ayurvedic formulary of India\textsuperscript{136} has also accepted this and is widely used for source of bala in northern parts of India. It is also used for medicinal purposes in the codified Indian
Siddha systems of medicine. It is stated in traditional siddha literature under the author Bhava Mishra, ‘Bhava Prakash Nigandu’ the roots of this plant used in liver diseases, rejuvenation and anti ageing\textsuperscript{137}.

Ethnobotanical Uses

It has a many medicinal properties and has been used by native peoples from all regions where it is found. In Vedic periods, the roots of the Bala plants \textit{i.e.} Atibala (\textit{Abution indicum} Linn.), Mahabala (\textit{Sida rhombifolia} Linn.), Bala (\textit{Sida cordifolia} Linn.) and Bhumibala (\textit{Sida veronicaefolia} Lam) were used to remove poison, vata – pitta diseases, heart problems, bily blood, eye diseases, and uterine disorders. Its seeds and roots both were used in fever in the form of decoction\textsuperscript{135-138}.

Summary of the Traditional Uses\textsuperscript{138-142}

\textit{Sida cordata} (Syn.-\textit{Sida veronicaefolia}) is very popular with rural womenfolk, especially in the areas where it grows in its natural habitat, and is used extensively in traditional medicine for shortening and reducing the pain of labour in childbirth. It is believed to render parturition almost painless and leads to shorter period of postpartum bleeding. Soup of this plant is taken in the last days of pregnancy.

\textbullet{} Fruits and Flowers: are refrigerant and are useful in relieving burning sensation, hyperdiuresis, pectorial lesions and promoting strength.

\textbullet{} Entire Plant: curing blood dysentery, fever, allergy and also aphrodisiac

\textbullet{} Leaf: Juice used in diarrhea, toothache, lumbago, piles, decoction of leaves is used in bronchitis, gonorrhea and inflammation of bladder, cut and wounds.

\textbullet{} Root: The roots are sweet, sour, astringent, bitter acrid thermogenic and are useful in fever uropathy and arthritis. The bark of root is used for leucorrhoea, gonorrhea and hyperdiuresis.
Phytoconstituents

The plants of genus *Sida* are very important. This importance is due to their chemical constituents. Phytochemically, these species contain a group of alkaloids like ephedrine and its isomers, vasicine, vasicinone and vasinol, due to which it has become as significant as Ephedra\textsuperscript{131, 143}. A detailed review of literatures revealed the presence of other various pharmacologically active phytoconstituents in the plant such as flavonoids, triterpenoids, phenolic compounds, saponins, amino acids and protein\textsuperscript{144}. Isolated chemical constituents includes β-phenethylamines, quinazoline, carboxylated trytamine, linoleic acid, malvalic acid, sterculic acid and gossypol and Sidaverin\textsuperscript{133, 145}.

**Scientific reports:**

*In vitro Hepatoprotective activity*

The ethanolic extract of whole plant *Sida cordata* was tested for hepatoprotective activity on the Chang cell line (normal human liver cells). The percentage viability of the cell line was carried out. The cytotoxicity of *Sida cordata* on normal human liver cell was evaluated by the SRB assay [Sulphorhodamine B assay] and MTT assay [(3-(4, 5 dimethylthiazole –2 yl)-2, 5 diphenyl tetrazolium bromide) assay]. The principle involved is the cleavage of tetrazolium salt MTT into a blue coloured derivative by living cells which contains mitochondrial enzyme succinate dehydrogenase. It was proposed to carry out a preliminary *in vitro* analysis of the hepatoprotective activity of the plant, which gave promising results\textsuperscript{146}.

**Immunomodulatory activity**

*Sida cordata* was evaluated experimentally for its immunostimulant properties. Experimental studies in rabbits showed that the entire drug treated groups of animals showed a higher level of anti-salmonella typhi ‘O’ titers. The values of titers were
statistically significant for 8\textsuperscript{th}, 16\textsuperscript{th}, and 24\textsuperscript{th} day, respectively when compared to the control group. Higher level of antibody titre was obtained on 24\textsuperscript{th} day of experiment. The protective effect of \textit{Sida cordata} against virulent \textit{staphylococcus aureus} challenge in the rabbits showed highly significant survival period (P<0.01). The haematological values for total and differential leucocytes count and Haemoglobin percent did not shown any particular change. However a significant decrease in Erythrocyte Sedimentation Rate levels (P<0.05) following \textit{Staphylococcus aureus} injection was noted in \textit{Sida cordata} treated animals. Histopathological studies of animals challenged with virulent \textit{staphylococcus aureus} showed marked protection of tissue damage\textsuperscript{147}.

\textbf{Adaptogenic activity}

A study was carried out to investigate mechanism of adaptogenic activity of a siddha medicinal plant, \textit{Sida cordata} (whole plant). Forced swimming test (FST) screening model used for evaluation of antidepressants / adaptogens activity. The experimental animals were euthanized and their brains were removed immediately, and the prefrontal cortexes (PFC) were dissected out on ice for biochemical analysis. The animals treated with total extract (100mg/kg) and (200mg/kg) showed significant decrease in the immobility period with simultaneous increase in antioxidant markers as well as adrenaline and serotonin levels. Study indicates positive adaptogenic activity of the extract \textit{Sida cordata} (whole plant) by forced swim test and resultant biochemical studies\textsuperscript{148}.

\textbf{Anti-inflammatory and antipyretic activity}

The study had been undertaken with a view to see the efficacy of the Bala and to explore the controversy. There are a number of source plants accepted in the name of bala in different parts of the country based on its predominant availability. Because Bala is the common drug mention in the almost all Ayurvedic formulation and widely

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described in all classical texts. Roots of all the four sources can be used for attaining anti-inflammatory and antipyretic effects and all the four sources were shown significant results, so it concluded that all the four source plants can be accepted for the drug Bala.\textsuperscript{149}

**Antioxidant activity**

The antioxidant activity of hexane, chloroform, hydro-alcoholic and aqueous extract of whole plant of *Sida veronicaefolia* (Syn. *Sida cordata*) was evaluated using in-vitro models, DPPH free radical scavenging, scavenging of hydrogen peroxide and reducing power method. Dried powder of whole plant was extracted with hexane, chloroform, hydro-alcohol (50%) and water using Soxhlet apparatus. The obtained results indicate that, the hydro-alcoholic extract of *Sida veronicaefolia* shows high scavenging activity. The antioxidant activity of the plant may be due to the presence of flavonoids, terpenoids and phenolic compounds of hydro-alcoholic extract of whole plant.\textsuperscript{150}

**Oxytocic effect**

Sidaverin, a crystalline compound extracted from a polar fraction of *Sida veronicaefolia* (Lam), elicited oxytocin-like contractions in the non-gravid rat isolated uterus preparation with a concentration-response relationship. Equipotent concentrations of oxytocin and sidaverin, using matched responses, were approximately 0.16 and 0.4 micrograms ml\textsuperscript{-1}, respectively. Sidaverin-induced contractile response was atropine reversible. The concentration-response curves for sidaverin and oxytocin were parallel, and both responses were inhibited by the specific oxytocin antagonist, Atosiban, indicating possible involvement of oxytocin receptors in the action of sidaverin. There was potentiation of action of one drug to that of the other, irrespective of the order of administration and even after washing off
the first before introducing the second drug. In the gravid uterus, sidaverin produced contractions in preparations from day 1st to day 6th or 7th, caused relaxation in days 7th -11th, and elicited contractions in day 11th through term, the sensitivity of the preparations increasing exponentially toward term with strong sustained contractions. With the exception of days 7th -11th, when sidaverin antagonized oxytocin action, it potentiated action of oxytocin on the gravid uterus151.

Abortifacient activity

Lutterodt reported that alcoholic extract of *Sida veronicaefolia* has abortifacient effect in pregnant rats. An oral dose produces abortifacient effect when administered from 15th-17th day of pregnancy152.

Anticancer activity

The acetone and ethanol extracts from the leaves of *Sida Veronicaefolia* i.e. AESV and EESV respectively were evaluated for antitumor activity against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. The extracts were administered at the doses of 500 mg/kg body weight per day for 14 days after 24 h of tumor inoculation. 24 h after the last dose, with fasting, the mice were sacrificed. The present study deals with the effect of AESV and EESV on mean survival time, tumor volume, tumor weight, tumor cell count, body weight, peritoneal cell count, haematological studies and *in vitro* cytotoxicity. AESV and EESV caused significant decrease in tumor volume, tumor weight, tumor cell count, body weight and it prolonged the life span *i.e.* mean survival time of EAC-tumor bearing mice and normal peritoneal cell count in normal mice. Haematological profile converted to more or less normal levels in AESV and EESV treated mice. AESV and EESV also exhibited significant cytotoxic activity at 200 µg/ml, but higher cytotoxic activity was
found in AESV. The results indicate that AESV and EESV exhibited significant antitumor activity in EAC-bearing mice\textsuperscript{153}.

**Anti-Microbial Activity**

Treatment with 70% alcoholic, 90% alcoholic, aqueous and chloroform extract of leaves of *Sida veronicaefolia* Lam. shows moderate zone of inhibition against both gram + ve and gram -ve bacteria and fungi\textsuperscript{154}.

**Antidiarrhoeal activity**

For castor oil, magnesium sulphate, prostaglandin E2 induced diarrhoeal models 70% alcoholic extracts shows significant antidiarrhoeal activity by reducing total no. of wet faeces, total weight of wet faeces and increase in the time elapsed between the administration of cathartic agents and excretion of the first diarrhoeic faeces. In addition gastrointestinal motility test 70% alcoholic extract significantly reduced gastric motility as indicated by the reduction in the distance travelled by charcoal meal\textsuperscript{154}.

**Gastro-protective activity**

For ethanol, indomethacin and pylorus ligation induced ulcer models 70% alcoholic extract showed significant gastro-protective activity by reducing mean ulcer index. Especially at 400mg/kg of 70% alcoholic extract of leaves of *Sida veronicaefolia* Lam. has given highest protection\textsuperscript{154}.
3.4 SCOPARIA DULCIS Linn.

Family: Scrophulariaceae

Synonyms: Scoparia grandiflora,
Scoparia ternata,
Capraria dulcis,

Vernacular Name:

Hindi: Mithi Patti, Ghoda Tulsi
English: Sweet broom, Broomweed, Vassourinha
Sanskrit: Asmaghni
Tamil: Sarakkoththini
Bengal: Bon dhonya

Scientific Classification:

Kingdom: Plantae – Plants
Sub kingdom: Tracheobionta – Vascular plants
Super-division: Spermatophyta – Seed plants
Division: Magnoliophyta – Flowering plants
Class: Magnoliopsida – Dicotyledons
Subclass: Asteridae
Order: Scrophulariales
Family: Scrophulariaceae – figwort family
Genus: Scoparia L. – licorice weed
Species: Scoparia dulcis L – licorice weed
Description:

*Scoparia dulcis* Linn. is a common annual herb in Suriname growing up to 2' in height. It has serrated leaves and many small white flowers. Flowers hermaphrodite, complete, usually axillary, 6-7 mm in diameters, 4-fid, rotate, regular. Sepals 4-5, gamosepalous, regular, calyx lobes oval-oblong, 2.5-3.0 X 0.8-1.0 mm, 3-nerved, glabrous, ciliate at margin, persistent. Corolla pale yellow to white, corona present, tube densely hairy at the throat, lobes 2-4 mm long, apex obtuse, slightly curvy, upper lobes slightly larger than others. Stamens 4, exerted; filament inserted at the top of the corolla tube, glabrous; anthers dorsifixed. Style erect, 2 mm long; stigma truncate to 2-partite, sometimes notched. Flowering time: Almost throughout the years\(^{155,156}\).

Distribution:

*Scoparia dulcis* is a perennial medicinal herb distributed throughout tropical and subtropical regions India, America, Brazil, West Indies, and Myanmar. It is introduced in India from Tropical America\(^{155,156}\).

Ethnobotanical Uses

In very country where *Scoparia dulcis* grows it hold a long history of use by indigenous peoples and herbalists. The traditional use of *Scoparia dulcis* has been recorded in herbal medicine system in the following parts of the world: Asia-Pacific\(^{157-159}\), Africa\(^{160}\), Central America\(^{161}\) and India\(^{155-166}\).

Summary of the Traditional Uses

**Aerial Parts:** Childbirth, coughs, diarrhea, expectorant, fever, stomachache.

**Entire Plant:** Abortive, aches, anemia, aphrodisiac, blennorrhagia, bronchitis, burns, childbirth, contraceptive, coughs, diabetes, diarrhea, dysentery, expectorant, fever, gastric disorders, headache, hemorrhoids, hepatosis, hypertension,
infections, insect bites, intestinal worms, jaundice, liver disease, malaria, menorrhagia, menstrual disorders, pain, rash, snake bites, swelling, toothache, venereal disease, wounds.

Leaf: Abortive, anemia, burns, childbirth, contraceptive, cough, diabetes, diarrhea, erysipelas, eye problems, fever, headaches, hemorrhoids, infections, insect bites and stings, intestinal worms, kidney disease, liver disorders, malaria, menstrual disorders, migraines, snake bites, stomach disorders, tonic, ulcers, urinary tract disorders, venereal disease, vomiting, wounds.

Root: Abortive, bronchitis, diarrhea, dysmenorrhea, fever, jaundice, liver disorders, malaria, menorrhagia, menstrual disorders, skin infections, stomach pains, warts.

Phytoconstituents

The scientific literature reveals numerous chemical studies on the herbs; isolated chemical constituents includes coumarins, phenols, saponins, tannins, amino acids, flavonoids, terpenoids and catecholamines\textsuperscript{167-169}. High-performance liquid chromatography analysis of an aqueous fraction of \textit{S.dulcis} revealed the presence of noradrenaline and adrenaline which have sympathomimetic effects\textsuperscript{170}. The herb's terpenoids are responsible for numerous medicinal effects. Scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin are biologically active. Additional identified terpenoids of broomweed includes alpha-amyrin, betulinic acid, dulcioic acid, friedelin, glutinol and ifflaonic acid\textsuperscript{161-175}.

The dried roots and aerial parts of \textit{Scoparia dulcis} contain economically important hydroxamic acids which provide insect, fungal and bacterial resistance\textsuperscript{169}. 
Scientific reports:

**Antidiabetic activity:**

Treatment with aqueous *S. dulcis* extracts and glibenclamide significantly improved specific insulin binding in streptozotocin-induced male wister rats. The number of insulin receptors and affinity binding (P<0.001) was reduced to normal non-diabetic level\(^{176}\).

**Analgesic and anti-inflammatory activity:**

The diterpene scoparinol demonstrated significant analgesic (P<0.001) and anti-inflammatory activity (P<0.01) in animals. Pretreatment of ethanolic extracts of *Scoparia dulcis* reduced acetic acid-induced writhing in mice 47%. The extract also inhibited paw edema in rats induced by carrageenan 46\%\(^{177}\).

**Anti-viral Activity**

*Scoparia dulcis* was investigated for anti-HSV-2 activity by plaque reduction assay. It was found that water extract of *S. dulcis* was active against HSV-2 with 50% effective dose of 1190.4\(\mu\)g/ml and ED\(_{50}\) of ethanol extract of *S. dulcis* was 13.8\(\mu\)g/ml. Ethanol extract of *S. dulcis* showed highest Therapeutic Index (TI) (2.9) against HSV-2G. The ethanol extract of *S. dulcis* was the best effective on HSV-2G using both plaque reduction assay and yield reduction assay\(^{178}\).

**Anti-malarial activity:**

*In vitro* the diterpenoid scopadulcic acid A has activity against various *Plasmodium falciparum* isolates with an IC\(_{50}\) of 27 mcM against the African Sierra isolate and IC\(_{50}\) of 19 mcM against the W\(_2\) clone (Indochina isolate)\(^{179}\).
Hepatoprotective Activity

The aqueous extract of *Scoparia dulcis* at a dose of 0.5 g/kg, *p.o.*, significantly prevents the CCl₄ induced prolongation of pentobarbitone sleep time, indicating the cytochrome P-450 protection activity¹⁸⁰.

Anti-cancer Activity

Scopadulcic acid B (SDB), a tetracyclic diterpenoid, inhibited the effects of tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) *in vitro* and *in vivo*; SDB inhibited TPA-enhanced phospholipid synthesis in cultured cells, and also suppressed the promoting effect of TPA on skin tumor formation in mice initiated with 7, 12-dimethylbenz[a]anthracene. The potency of SDB proved to be stronger than that of other natural antitumor-promoting terpenoids, such as glycyrrhetinic acid¹⁸¹.

Wound healing Activity

Leaves are the most frequently utilized plant part and most herbal remedies are prepared as paste and applied externally; in some cases medicinal preparations are also administered orally. *Scoparia dulcis* has been reported to have such specific wound healing compounds¹⁸².

On Tissue Antioxidant Defense System

Antioxidant activity of *Scoparia dulcis* extracts had been determined by estimation of DPPH radical and nitric oxide radical scavenging activity. Hydroalcohol extract of *Scoparia dulcis* L. showed a significant antioxidant activity than other extracts which reduces the Nitric oxide radical and DPPH radical up to an extent of 0.124 and 0.11 at a concentration of 500µg/ml of the extract¹⁸³.