



A Review on Hepatoprotective Activity of Citrus Limetta

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ABSTRACT

Hepatic diseases are a major worldwide health problem, with frequently found in developing countries. They are mainly caused by uses of high doses of chemicals and some drugs. There is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells. There-fore there is urgent need, for effective drugs to replace/add those in current use. Medicinal herbs are significant source of pharmaceutical drugs. Latest trends have shown increasing demand of phytoconstituents from some medicinal herbs and those medicinal herbs have proven hepatoprotective potential. A number of herbal preparations are available in the market. The present review is aimed at compiling data on promising phytochemicals from medicinal plants that have been tested in hepatotoxicity models using modern scientific system. In this century clinical research has confirmed the efficacy of some herbs in the treatment of liver related disease. Hence, this review article contributes to the knowledge of reported indigenous plants, which are prevalent for prevention and treatment of liver disorders.

Key Words: Hepatic diseases, Hepatoprotective potential, Phytoconstituents, Hepatotoxicity models.

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INTRODUCTION

Hepatoprotective agents are those compounds, which moderate the liver injury cause by hepatotoxic agent. Hepatoprotective result of plant medicine & herbal formulation are studied against chemicals and drug induced hepatotoxicity in rat and mice as they virtually mimic any form of naturally occurring liver disease. A variety of herb such as Catharanthus tinctorius, Tamarind indica, homalomena aromatica, Wedelia calendulacea, Punica granatum, Malus domestica, Solanum lycopersicum use like hepatoprotective agent.

According to WHO (2002) information, about 80% of the world population relies on traditional organization of medicine for major physical condition concern, where plants from the leading part over the natural resources; exclusively developing countries like India which expansively used substitute medicines for healthcare. The conventional medicine refers to a broad variety of early healthcare practices as well as Ayurveda, Siddha and Unani. Henry James assured that “it takes an incessant quantity of history to create even a small custom”. This statement fits well for the training of traditional medicine because each one of it is the result of numerous trials approved out for a long period of time in humans.

PLANT PROFILE

Figure: 1 *Citrus limetta*

<i>Citrus limetta</i>	
Scientific classification	
Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Rutaceae
Genus:	Citrus
Species:	<i>C. limetta</i>

Description



(a)



(b)

Figure: 2 (a) The limetta (lemetjie) , Mosambi cultivar, at a market in Seethammadhara. Figure: 2 (b) Mosambi (sweet lime) juice is a favorite citrus drink in India

C. limetta is a small tree up to 8 m (26 ft) in height, with irregular branches and relatively smooth, brownish-grey bark. It has numerous thorns, 15–75 mm (0.59–2.95 in) long. The petioles are narrowly but distinctly winged, and are 8–29 mm (0.31–1.14 in) long. Leaves are compound,

with acuminate leaflets 50–170 mm (2.0–6.7 in) long and 28–89 mm (1.1–3.5 in) wide. Flowers are white, 20–30 mm (0.79–1.18 in) wide. Fruits are oval and green, ripening to yellow, with greenish pulp. The pith is white and about 5 mm (0.20 in) thick. Despite the name sweet lime, the fruit is more similar to a greenish orange in appearance.



Figure: 3 'Millsweet' cultivar of limetta in growth.

C. limetta grows in tropical and subtropical climates. It begins bearing fruit at 5 to 7 years old, with peak production at 10 to 20 years. It is propagated by seed.

Flavour



Figure: 4 Freshly pressed sweet lime juice as served in the Indian subcontinent

As the name *sweet lime* suggests, the flavour is sweet and mild, but retains the essence of lime. The lime's taste changes rapidly in contact with air, and will turn bitter in few minutes, but if juiced and drunk rapidly the taste is sweet. The flavour is a bit flatter than most citrus due to its lack of acidity. It can be compared to limeade and pomelo.

Uses

Sweet lime is almost exclusively served as juice, and is the most common available citrus juice in the Indian

subcontinent. The juice is commonly sold at mobile road stalls, where it is freshly pressed, sometimes served with a salty chat masala or kala namak, unless the vendor is told not to add it.

Like most citrus, the fruit is rich in vitamin C, providing 50 mg per 100 g serving.^[7] In Iran it is used to treat influenza and common cold.

The tree is used for ornamental purposes as well as for graft stock.^[8]

Sweet Lime	
Nutritional value per 100 g (3.5 oz)	
Energy	180 kJ (43 kcal)
Carbohydrates	9.3 g
Sugars	1.7g
Dietary fibre	0.5 g
Fat	0.3 g
Protein	0.7-0.8 g
Vitamins	Quantity %DV [†]
Vitamin C	60% 50 mg
Minerals	Quantity %DV [†]
Calcium	4% 40 mg
Iron	5% 0.7 mg
Phosphorus	4% 30 mg
Potassium	10% 490 mg

Checking for ripeness

Like most citrus, sweet limes will not ripen off the tree, and must be picked when fully ripe. This is indicated by its tennis ball size and lustrous greenish yellow sheen. Gently scratch the surface of a sweet lime: If its oils give way in the fingernails, it is ripe. The juiciest fruits feel heavy for their size.

Underripe fruit feels light for its size, and is hard with tart flesh. Overripe fruit is dull and shrunken, with dry, spongy skin. Avoid fruit with brownish-yellow discoloration.^[9]

Storage

Sweet limes keep fresh for up to two weeks at room temperature, and four to eight weeks refrigerated. Frozen juice will keep for up to six months. It is possible to freeze slices of the fruit, though the limonin content may cause the pulp to taste bitter over time. This can be avoided by submerging the slices in sweet syrup within an airtight glass jar.

HEPATOTOXICITY:

Hepatotoxin is atoxic chemical substance which damages the liver. Toxic liver injury produced by drugs and chemicals may virtually mimic any form of naturally occurring liver disease. Hepatoprotective effect was studied against chemicals and drugs induced hepatotoxicity in rats like alcohol, carbon tetrachloride, galactosamine, paracetamol, isoniazid and rifampicin, antibiotics, peroxidised oil, aflatoxin etc.

Severity of hepatotoxicity is greatly increased if the drug is continued after symptoms develop. Among the various inorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. The organic agents include certain naturally occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins.

Liver injury caused by hepatotoxins, such as carbon tetra chloride (CCl₄), ethanol and acetaminophen, is characterized by varying degrees of hepatocyte degeneration and cell death via either apoptosis or necrosis. The generation of reactive intermediate metabolites from the metabolism of hepatotoxins and the occurrence of

reactive oxygen species (ROS) during the inflammatory reaction, account for a variety of pathophysiologic Pathways leading to cell death, such as covalent binding, disordered cytosolic calcium homeostasis, GSH depletion, onset of mitochondrial permeability transition (MPT) and associated lipid peroxidation. The metabolism of hepatotoxins by cytochrome P-450 enzyme subtypes is a key step of the intoxication; therefore, enzyme inhibitors are shown to minimize the hepatotoxin-associated liver damage. Moreover, substantial evidence exists that MPT is involved in ROS-associated hepatocellular injury and new findings offer a novel therapeutic approach to attenuate cell damage by blocking the onset of MPT. Thus, oxidant stress and lipid peroxidation are crucial elements leading to hepatotoxin-associated liver injury. In addition to specific treatment for a given hepatotoxin, the general strategy for prevention and treatment of the damage includes reducing the production of reactive metabolites of the hepatotoxins, using anti-oxidative agents and selectively targeting therapeutics to Kupffer cells or hepatocytes for on-going processes, which play a role in mediating a second phase of the injury⁴.

CLASSIFICATION OF HEPATOTOXINS:

A. Intrinsic

It consists of agents that are predictable hepatotoxins. They are recognized by high incidence of hepatic injury exposed individuals and in experimental animals. There is a consistent latent period between exposure to a particular agent and the development of hepatic injury and the injury appeared to be dose related^{5,6}. There are two types of intrinsic hepatotoxins:

Direct hepatotoxins:

It may be so called because they (metabolic products) produce direct injury to hepatocytes and its organelles, especially the endoplasmic reticulum. CCl₄, the prototype, produces peroxidation of the membrane lipids and other chemicals that lead to degeneration of the membranes.

Indirect hepatotoxins:

They are anti-metabolites and related compounds that produce hepatic injury by interference with the specific metabolic pathway or processes. The structural injury produced by indirect hepatotoxins, appear to be secondary to a metabolic region. While in that produced by direct hepatotoxins, the metabolic disarrangement is secondary to the structural injury. The hepatic damage produced by indirect hepatotoxins may be mainly cytotoxic injury (by interfering with metabolic pathway or processes essential for parenchyma integrity) expressed as steatosis or necrosis, or may be mainly cholestasis, interfering only or mainly with biliary secretion.

B. Host idiosyncrasy:

It consists of agents that are not predictably hepatotoxic, but produces hepatic injury in only a small portion of exposed individual. In several instances auto antibodies directed against normal cellular constituents are detected. The injury does not appear to be dose related and is not reproducible in experimental animals and appears after a variable latent period.

Evaluation of Hepatoprotective Activity:

A review of literature reveals that several chemical substances and drugs having specific actions on liver are used as hepatotoxins in experimental animals to simulate ideal diseased conditions. The hepatoprotective activity can be most easily evaluated /screened with the aid of several model systems of liver damage in experimental animals.

In all test model systems, conditions for liver damage are implemented and an attempt is made to counteract this toxicosis with the substance/preparation under test. The magnitude of the protective effect can be measured by estimating the enzyme activities and the rate of survival and can be verified histologically. The available methods are in vivo, ex vivo and in vitro methods⁸. All these methods are used to study the protective or curative effects of any compound under test. In order to test for hepatoprotective activity the test substance and the hepatotoxin are administered simultaneously whereas in case of antihepatotoxic or curative activity the test substance is generally administered after induction of hepatotoxicity

In- vitro methods:

Hepatocytes are generally isolated by using in-situ, two step recirculating collagenase perfusion technique. These are then seeded in small containers and exposed to test samples and toxins. After a specified time period, the degree of toxicity or protection is assessed by viability tests and enzyme levels such as GOT and GPT. By employing primary culture hepatocytes using CCl₄, galactosamine, thioacetamide, ethanol, paracetamol (PCML) etc. as hepatotoxins several hepatoprotective screening models have been devised. These have a number of advantages over in vivo methods such as their ability to dispose numerous samples at a time, low cost with a small size, little variation and reproducibility of results. The major disadvantage is that sometimes it may not reflect the events which occur in animals.

Ex- vivo models:

In this model, after completion of preselected in vivo test protocol hepatocytes are isolated and the percentage of viable cells and biochemical parameters are determined as liver function tests. These methods are somewhat better correlated to clinical models than in vitro or in vivo methods.

In- vivo methods:

This method is used not only to study the nature of the given compound but also to study the mechanism of the toxicant. Hepatotoxicity is produced in experimental animals by the administration of known dose of hepatotoxins like CCl₄, galactosamine, thioacetamide, ethanol and paracetamol etc., which produce marked measurable effects, the magnitude of which can be measured by carrying out various liver function tests viz. morphological, metabolic or functional, biochemical and histopathological determinations. Although it is a very convenient laboratory method, reproducibility of results is rather poor. The compounds having hepatoprotective claims are also evaluated in general for their choleretic or anticholestatic activity in order to know whether the liver disorder is due to an abnormality of bilirubin metabolism or not. Choleretics are those agents which increase the output of bile by stimulating the liver whereas anticholestatics are those which correct the retention and accumulation of bile due to intrinsic and extrinsic factors in the liver. These activities are evaluated by studying bile flow content in conscious and anaesthetized animals for 5 hours.

Experimental Models for Hepatoprotective Screening:

Several chemical reagents and drugs which induce necrosis, cirrhosis, carcinogenesis and hepatobiliary dysfunctions in experimental animals are classified as hepatotoxins. The following are some of the experimental models explained by employing some of the important hepatotoxins.

CCl₄ model:

A number of CCl₄ models are devised depending upon its dosage through different routes of administration.

Acute hepatic damage: Acute liver damage, characterized by ischemia, hydropic degeneration and central necrosis is caused by oral or subcutaneous administration of CCl₄ (1.25ml/kg). The maximum elevation of biochemical parameters is found to be 24 hours after the CCl₄ administration normally administered as 50% v/v solution in liquid paraffin or olive oil⁹.

Chronic reversible hepatic damage: Administration of CCl₄ (1ml/kg S.C.) twice weekly for 8 weeks produces chronic, reversible liver damage.

Chronic, irreversible hepatic damage: Administration of CCl₄ (1ml/kg S.C.) twice weekly for 12 weeks simulates chronic, irreversible liver damage.

Thioacetamide model: Thioacetamide (100mg/kg s.c.) induces acute hepatic damage after 48 hrs of administration by causing sinusoidal congestion and hydropic swelling with increased mitosis¹².

D-galactosamine model: D-galactosamine (800mg/kg i.p.) induces acute hepatotoxicity after 48 hrs. of administration with diffused necrosis and steatosis.

Paracetamol model:

Paracetamol induces acute hepatotoxicity depending upon its dosage through different routes of administration, such as Paracetamol (800mg/kg i.p.) induces centrilobular necrosis without steatosis. Paracetamol at a single dose of 3g/kg p.o. stimulates acute hepatic damage. It takes 48 hrs to induce the toxicity.

Chloroform model:

It produces hepatotoxicity with extensive central necrosis, fatty metamorphosis, hepatic cell degeneration and necrosis either by inhalation or by subcutaneous administration (0.4-1.5ml/kg).

PARACETAMOL INDUCED HEPATOTOXICITY

Paracetamol or acetaminophen (N-acetyl-P-aminophenol, APAP) induced hepatic injuries are commonly used models for screening of hepatoprotective drugs and it causes acute centrilobular necrosis in rats, mice, guinea pig, hamsters, rabbits, cats, dogs and pigs and centrilobular hemorrhagic necrosis in humans mostly characterized by pyknosis and eosinophilic cytoplasm.

Paracetamol is one of the most widely used drug for analgesic and antipyretic activity worldwide. It is one of the most common pharmaceutical associated with both intentional and accidental poisoning. It is a major cause of liver failure and causes death when taken in excess and is assumed to be safe in recommended doses. It produces hepatic necrosis at higher doses. Paracetamol is rapidly absorbed from the stomach and small intestine and metabolized by conjugation in the liver to non-toxic agents. Therapeutic doses of drug are metabolized mostly to sulphate and glucuronide conjugates. The rest is metabolized to a reactive intermediate which is detoxified by conjugation with glutathione. In acute overdose or when the maximum daily dose is exceeded over a prolonged period, the normal conjugative pathway of metabolism

becomes saturated. Excess paracetamol is then oxidatively metabolized in the liver via the mixed function oxidase P450 system to a toxic metabolite N-acetyl-P-benzoquinonimine (NAPQI). NAPQI has extremely short half-life and is rapidly conjugated with glutathione, a sulfhydryl donor. Under conditions of excessive NAPQI formation or reduced glutathione store, NAPQI covalently binds to vital proteins and the lipid bilayer of hepatocyte membranes. The result is hepatocellular death and centrilobular liver necrosis. Small doses are eliminated by conjugation followed by excretion but when the conjugation enzymes are saturated, the drug is diverted to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative by cytochrome P450 enzyme. The hydroxylamine derivative, a reactive electrophilic agent, reacts non-enzymatically with glutathione reacts with macromolecules and disrupts their structure and function. Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome P450 or depletion of hepatic glutathione is a prerequisite for paracetamol-induced toxicity. In over dose, the sulphate and glucuronide conjugation pathways are saturated and more drugs are converted to the reactive metabolite. The glutathione available for its detoxification is rapidly depleted and the metabolites accumulated bind covalently to liver cell proteins, causing irreversible damage. Liver damage can be prevented by providing glutathione like substances, such as acetylcysteine, so that the reactive metabolite can be removed by conjugation and the liver cells are protected.

An alternative view is that oxidative stress has a role in hepatotoxicity. There are many characteristic features of oxidative stress in APAP hepatotoxicity, including lipid peroxidation, mitochondrial damage, ATP depletion, and formation of nitro tyrosine adducts in proteins, presumably owing to formation of superoxide-derived peroxynitrite. However, these processes may be consequences of the damage mediated by protein adduction rather than the direct effect of hepatotoxicity.

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