LITERATURE SURVEY
Chapter 2

2.0 LITERATURE SURVEY

2.1 PHARMACOGNOSY OF PIPER LONGUM LINN

Medicinal plants have been successfully used for the treatment of many serious diseases. Since heart diseases are affecting millions of people throughout the world, it becomes necessary to find out alternative treatments, new drug molecules for the effective treatment of heart diseases. Many plants and plant derived products have shown useful activities against heart diseases.

In the present study the roots of the plant *Piper longum linn* (Family: *Piperaceae*) was selected for finding out its usefulness for the treatment of heart diseases.

The exact role of the plant has not been mentioned or studied in detail. The aim of the present studies was hence to find out the exact role of plant and its constituents in the heart diseases.

Long pepper, which tastes pungent and sweet at the same time, probably came to Europe much before now dominant black pepper. Its hot-and-sweet taste goes well with spicy cheese specialties or wine sauces. In India, the long pepper is mainly used in pickles (achar).

*Cultivation*

Pepper long is cultivated on a large scale in lime stone soil and in heavy rainfall areas where relative humidity is high. Long pepper is also known and popular in parts of Africa, mostly in the Islamic regions of North and East Africa. It can be found in the complex spice mixtures of Morocco. It is also of some importance for the cuisine of Ethiopia, where long pepper is usually found in the traditional meat stews (wat) together with black pepper, nut meg, cloves and turmeric.

*Uses*

Aromatic, stimulant, carminative, good for constipation, for gonorrhea, paralysis of the tongue, advised in diarrhea, cholera, scarlatina, chronic malaria, viral hepatitis. *Piper longum linn* is most commonly used to treat respiratory infections such as stomachache, bronchitis, diseases of the spleen, cough, tumors, and asthma. When applied topically, it soothes and relieves muscular pains and inflammation. In Ayurvedic medicine, it is said to be a good rejuvenator. *Piper longum linn* helps to stimulate the appetite and it dispels gas from the intestines. An infusion of *Piper longum linn* root is used after birth to induce the expulsion of the placenta.

Pepper contains volatile oil, the crystalline alkaloids: piperine, piperidine, piperettine, and a resin. Long pepper contains the alkaloid piperine (about 6%), which is slightly higher than that in black pepper. *Piper longum linn* is widely used in Ayurvedic and Unani systems of medicine particularly for diseases of respiratory tract most of them includes cough, bronchitis, asthma etc.
Long pepper is locally applied to counter-irritant and act as analgesic for muscular pains and inflammation. Long pepper acts as a general tonic and hematinic and widely used in Ayurveda as good rejuvenator (Rasayana). *Piper longum* is known to enhance the bio-availability of food and drugs. In fact *Piper longum* is taken along with Quinine for making it more effective. *Piper longum* is widely used as a carminative.

Other Uses: *Piper longum* is used as a spice and also in pickles and preserves.

**Description**
The erect shrub has a thick, jointed and branched root-stock. Leaves are numerous, 6.3 to 9.0 cm, broadly ovate or oblong-oval, dark green and shining above, pale and dull beneath. Fruits are present in a solitary, pedunculate, fleshy spike 2.5 to 3.5 cm long, 5 mm thick, ovoid, oblong, erect, blunt, blackish green in colour and shining. Odour is aromatic and the taste is pungent.

**Characteristics and Constituents of Piper longum**
The fruits contain 1% volatile oil, resin, alkaloids piperine and piperaluminine, a waxy alkaloid N-isobutyldeca-trans-2-trans-4-dienamide and a terpenoid substance. Roots contain piperine, piperalumin or pipiplartine, dihydrostigmasterol. Its principal constituents are piperine and pipiplartine. *Piper longum*’s Ayurvedic or Sanskrit name is “Pippali”, and in the West it is known as Long pepper. It belongs to the Piperaceae family and it is the fruits and roots, which are used medicinally. Rasa (taste) is katu (pungent), Virya (energy) is ushna (hot) and Vipak (post digestive action) is madhura (sweet).

The berries are a cardiac stimulant, carminitive, tonic, laxative, digestive, stomachic and antiseptic. It is a mild diuretic, alterative, hepatic and expectorant.

The fruit contains volatile oil, starch, protein, alkaloids-piperine and piperaluminine, saponins and lignans. Pippali, like its relative Black pepper, is a powerful stimulant for the digestive and respiratory systems. It is strongly heating, removes colds, congestion and toxins and revives weak organ functions.

Black pepper (*Piper nigrum*) and long pepper (*Piper longum*) are the best known species in this family and are probably among the most recognized spices in the world. Black pepper alone accounts for about 35% of the world’s total spice trade. In addition, black pepper and long pepper have been used medicinally for centuries. In recent years, extensive research data on the phytochemistry and unique pharmacological actions of these plants have also become available. The Materia Medica of Ayurveda, which dates back to 6,000 B.C., has many references advocating the use of pepper in a variety of ailments, particularly those pertaining to the gastro-intestinal tract. The earliest travelers from Europe who visited India described pepper cultivation on the Malabar coast. Theophrastus mentions two kinds
of pepper in the fourth century B.C., (most likely these were black pepper and long pepper). Discorides in the first century A.D. mentions black pepper and long pepper as well as white pepper, which is simply black pepper seed with its peel or pericarp removed. Black pepper and long pepper were among the spices from India on which the Romans levied import duty at Alexandria, around A.D. 176. Pepper is mentioned by Roman writers in the fifth century A.D. It is said that Attila the Hun demanded, among other items, 3,000 lbs. of pepper as ransom for the city of Rome. Centuries later, the high cost of pepper led the Portuguese to seek their own sea passage to India. The Portuguese were successful in this mission and monopolized the spice trade until the 18th century. In January 1793, an agreement was made between the Rajah of Travancore and the Crown of England. The Rajah was to supply large quantities of pepper to the Bombay Government in return for arms, ammunition and European goods. This is known historically as the “Pepper Contract”. In traditional Chinese medicine, black pepper has been used for the treatment of epilepsy. Based on this traditional application, a new antiepileptic drug called Antiepilepserine has recently been synthesized by Chinese researchers [1].

Black pepper

From the 16th to the 18th centuries, the struggle for control of the Far Eastern spice producing regions became so intense that wars were fought between Portugal, Holland, and England. By the end of the 18th century, the United States entered the world trade for spices, bartering its salmon, flour, and soap for tea, coffee and spices. One reason spices in general, and pepper in particular, became so important in international trade was their popular culinary role. In those times, tough, heavily salted long-stored meat was standard fare, and spice additives made these meats more palatable, while simultaneously masking off-flavors.

Cultivation

Members of the botanical family Piperaceae were among the first cultivated plants. Long pepper is successfully cultivated in well-drained forest soils rich in organic matter. Laterite soils with high organic matter content and moisture holding capacity are also suitable for cultivation. Areas with high rainfall and high humidity with an elevation of 100-1000 m is ideal. It grows well under semi-shady conditions (25-50 per cent shade) in irrigated coconut gardens.

Propagation is through suckers or rooted vine cuttings, 15-20 cm long with three-five cm nodes. March-April is the best time for raising nursery. The rooted cuttings will be ready for transplanting in two months. With the onset of monsoon in June, the field is ploughed well and raised beds of convenient length and breadth are taken. On these beds, pits are dug at 60 x 60 cm spacing and well-decomposed organic manure at the rate of 100 g/pit is applied and mixed with soil. Rooted vine cuttings are then transplanted to these pits. Crop growth and spike production increases by the
application of wood ash. It is reported that unirrigated crop after the onset of monsoon grows vigorously and shows much hardiness that the irrigated crop. A study conducted at Kerala Agricultural University to find out the optimum spacing and manorial recommendation revealed that plant height, number of branches, number of leaves and total dry matter increased with high dose of organic manure with an optimum spacing of 50 x 50 cm. In soils with low fertility the growth of the plant is very poor.

The pests like mealy bugs and root grubs, attack the plant particularly during summer, which can be controlled by drenching with systemic insecticides like nuvacron or dimecron. The vines start flowing six months after planting and flowers are produced almost throughout the year. The spikes are harvested when they are full-grown but yet unripe and become blackish green in colour and then dried in sun for four-five days. If left without picking they ripe and their pungency is lost to a great extent. The yield of dry spike is 400 kg/ha during first year, increases to 1000 kg/ha in the third year and decreases there after. The green to dry spike ratio is 10:15 by weight. After the third year, the whole plant is harvested. The stem is cut close to ground and roots are dug up. Average yield is 500 kg dry roots/ha. Stems and roots are cleaned, cut into cylindrical pieces of 2.5-5 cm length and 0.5-2.5 mm thickness, dried in shade and marketed as piplamool.

**Habitat**

*Piper longum linn* is a slender aromatic climber with perennial woody roots. The stems are jointed; the leaves, ovate and cordate with broad rounded lobes at the base, entire and glabrous; the spikes, cylindrical, male spikes larger and slender; the fruits, ovoid and yellowish orange.

It occurs in the hotter parts of India, from the Central Himalayas to Assam, the Khasi and the Mikir hills, the lower hills of Bengal and the evergreen forests of the Western Ghats from Konkan to Travancore. It has also been seen growing in the Car Nicobar Islands.

Whole plant and parts of plant like fruit and roots of the plant *Piper longum linn* (Family: Piperaceae) have been has been traditionally used for the treatment of various diseases[2].

Species of the piper have been used as spicy food material since ancient times. Among the different species of piper as *Piper nigrum*, *Piper longum linn* and *Piper retrofractum vahl* is widely used as spice all over the world. The medicinal values of piper species are well documented in the ancient literature of asian countries. Indian long pepper is indigenous to north-eastern and southern parts of India and Srilanka. In the western part of India aqueous extract of the roots of *Piper longum linn* are used as food material.

Fruits of the plant have been used as stomachic, aphrodisiac, thermogenic, carminative, expectorant, digestive, emollient, anti-girdiasis, anti-amoebic and
antiseptic [3-4]. The literature data shows that the roots of the plant have been used for the treatment of heart diseases. The benzene extract of fruits of *Piper longum linn* when mixed with the methanolic extract of the *Embelia ribes* berries and administered to female rats showed 80% of inhibition of pregnancy, suggesting its anti-fertility activity [5]. In another study, 20 pediatric patients with asthma received long pepper in doses ranging from 9.35 to 15.75 gm daily for several weeks. As a result of this treatment all patients showed clinical improvement. Dry fruits of *Piper longum linn* have been used in the prevention of recurrence of asthma [6]. *Piper longum linn* is effective in gastric ulceration when given along with the extracts of *Zingiber officinalis* and Ferula species [7].

Anti-inflammatory activity [8] of *Piper longum linn* was observed in Brahmi Rasayana. extracts of *Piper longum linn* showed immunostimulatory activity in mice. Along with *Butea monosperma* extracts of the plants *Piper longum, Glycyrrhiza glabra, Terminalia chebula* and shankha bhasma has found to increase beta glucoronidase activity of brunners glands in duodenal ulcers of rats [9]. The plant shows immunomodulatory activity [10]. Extracts of the plant also shows hepatoprotective action against carbon tetrachloride induced liver damage [11].

Phytochemical investigation of the plant showed presence of Piperonlongumine, Piperonlonguminine, Piperine, Sesamin, 3,4,5-trimethoxyccinnamate, beta-Sitosterol, Aristolactum, Piperyline, Pipartine and Hexacosanoic acid isobutyl amide [12-13]. Alkaloidal amides of the plant shows enhancement in the bioavailability of therapeutically important drugs [5]. Amides isolated from *Piper longum* shows diverse pharmacological and biochemical properties [14]. *Piper longum linn* (Piperaceae) is also widely used as a folk medicine to cure diseases such as leprosy and tuberculosis. Alkaloidal amide *Piper longum linn* shows increase in the bioavailability of various therapeutically diverse drugs. Species of the piper have been used as spicy food material since ancient times.

### 2.2 Piperine as Major Constituent of *Piper longum linn*

Piperine (1-piperoylpiperidine) is an alkaloid and the main pungent principle in both Black and Long pepper. Piperine is a solid substance essentially insoluble in water. It is a weak base that is tasteless at first, but leaves a burning aftertaste. Piperine belongs to the vanillloid family of compounds, a family that also includes capsaicin, the pungent substance in hot chili peppers. Its molecular formula is C_{17}H_{19}NO_{3}, and its molecular weight is 285.34 daltons. Piperine is the trans-trans stereoisomer of 1-piperoylpiperidine. It is also known as (E, E)-1-piperoylpiperidine and (E, E)-1-[5-(1, 3-benzodioxol-5-y1)-1-oxo-2, 4-pentdienyl] piperidine. It is represented by the following chemical structure:
The biological properties of piperine have been extensively studied only in recent years. The proposed mechanism for the increased bioavailability of drugs coadministered with piperine is attributed to the interaction of piperine with enzymes that participate in drug metabolism, such as mixed function oxidases found in the liver and intestinal cells. Interaction with the synthesis of drug chelating molecules in the body such as glucuronic acid has also been proposed. Piperine may also interact with the process of oxidative phosphorylation, or the process of activation/deactivation of certain metabolic pathways, slowing down the metabolism and biodegradation of drugs. This action of piperine results in higher plasma levels of drugs, rendering them more available for pharmacological action.

In pharmacological studies in animals, piperine has been shown to stimulate digestive enzyme activity, including that of pancreatic lipase, amylase, trypsin and chymotrypsin [15], as well as intestinal lipase, sucrase and maltase [16].

In one pharmacological study, piperine was found to protect against gastric ulceration; it also inhibited gastric acidity and pepsin A activity [17].

Piperine has been found to affect the cytochrome P-450 family of metabolising enzymes, and both stimulation and suppression of specific enzymes have been demonstrated in different models [18-19]. Piperine has also been shown to inhibit another metabolic step, glucuronidation, in isolated intestinal cells [20].

Despite the contrasting findings in terms of the effect of piperine on the cytochrome P-450 enzymes, most human and animal studies indicate that piperine inhibits, rather than stimulates, drug metabolism in most cases, thus increasing the bioavailability and effect of some drugs.

As will be further discussed below, piperine is chiefly responsible for the effect of Trikatu and peppers of increasing the bioavailability of many other compounds. In animals, piperine has demonstrated anti-convulsive activity [21-22], CNS stimulating and depressant activity, as well as anti-pyretic, analgesic and anti-inflammatory effects.

In addition, piperine has been shown to interact with the serotonergic system and deplete substance P in the spinal cord, and neuromuscular transmission and sensory receptors are reported to be affected by the compound.
Anti-fertility action but also pro-fertility effects of piperine have been observed in various animal models [23-27].

Piperine is almost completely (97%) absorbed from the gastrointestinal tract following oral administration in rats, and after 24 hours only traces remained in serum, kidney and spleen. In rats, the metabolism of piperine appears to involve glucuronidation and sulfation pathways, and metabolites include vanillic and piperonal acid, piperonylic acid and piperonyl alcohol [28].

There are in vitro, animal and human studies demonstrating that piperine can significantly increase the bioavailability of numerous drugs and some nutritional supplements. Reportedly, it has demonstrated this effect with some antimicrobial, antiprotozoal, antihelminthic, antihistaminic, non-steroidal anti-inflammatory, muscle-relaxant and anticancer drugs, among others. It has also increased the bioavailability of coenzyme Q10, curcumin and beta-carotene.

Enhanced bioavailability as a result of co-administration with either piperine, Black pepper or Long pepper, or Trikatu has been observed for several drugs including vasicine (from Adhatoda vasica), sparteine (from Spartium junceum) but also found in broom, (Cytisus scoparius), phenytoin, propranolol, theophylline, pentobarbitone, sulphadiazine and tetracycline [29-30].

Piperine administered at a dose of 30 mg/kg together with sparteine to rats more than doubled the bioavailability of sparteine [2].

A study on human volunteers found that the bioavailability of theophylline (150 mg) or propranolol was increased by the concurrent administration of 20 mg piperine daily for 7 days, as evidenced by higher Cmax and AUC for both compounds and longer elimination half-life for theophylline [30].

In the case of the anti-tuberculosis drug, rifampicin, several studies have yielded inconclusive results. The bioavailability of rifampicin has been found to increase in patients with pulmonary tuberculosis when co-administered with piperine, while Trikatu had been found to decrease the bioavailability of rifampicin in healthy volunteers.

The primary mechanism by which piperine (and Trikatu) affects the bioavailability of other compounds seems to be via the nonspecific and noncompetitive inhibition of microsomal liver metabolism involving the cytochrome P-450 enzyme system [31].

Piperine has been shown to inhibit certain hydroxylation, demethylation, deethylation and glucuronidation metabolic processes, and in vitro studies with hepatic microsomal suspensions have found that piperine inhibited a variety of mixed function oxygenases [29].

It has also been suggested that piperine could interact with intestinal epithelial cells with increased absorption as a result, and in vitro studies have shown piperine to stimulate gamma-glutamyl transpeptidase activity and increase amino acid uptake by
rat jejunum epithelial cells. These findings suggest that piperine may cause increased permeability of intestinal cells [14].

The pharmacokinetics of piperine in humans remains incompletely understood. In rats, piperine is absorbed following ingestion, and some metabolites have been identified: piperonylic acid, piperonyl alcohol, piperonal and vanillic acid are found in the urine. One metabolite, piperic acid, is found in the bile. Human pharmacokinetic studies are needed.

In humans given 2-gram doses of curcumin alone, levels of curcumin in serum were undetectable to very low one hour post-administration. Concomitant administration of 20 mg of piperine was said to significantly increase absorption and bioavailability (by 2000%). Similar results were reported in rats.

In a double-blind crossover study [32], 5 mg of piperine daily for 14-day periods resulted in significant increases in serum beta-carotene levels. The same dose of piperine produced similar results in another study, this one involving coenzyme Q10.

The claim that piperine may aid in the digestion of food is based on some experimental animal data showing that dietary piperine seems to enhance pancreatic amylase lipase, trypsin and chymotrypsin activity.

The claim that piperine may have some anticonvulsant activity [33] comes, in part, from China, where the substance is used in an effort to treat some forms of epilepsy. In mice, piperine injected intraperitoneally inhibited clonic convulsions induced by kainate. It did not significantly block seizure activity induced by L-glutamate, N-methyl-D-aspartate or guanidinosuccinate.

In a rat intestinal model, piperine was said to provide protection against oxidative changes induced by a number of chemical carcinogens [34]. In another study, this one in vitro, piperine reportedly reduced the cytotoxicity of aflatoxin B1 in rat hepatoma cells.

Piperine exhibited significant anti-inflammatory activity [35] in carageenan-induced rat paw edema and in some other experimental models of inflammation. In one animal study, piperine reduced liver lipid peroxidation, acid phosphatase and edema induced by carageenan.

On the negative side, piperine has shown some evidence of being mutagenic and potentially carcinogenic under some circumstances [36]. It has reportedly given rise to mutagenic products on reaction with nitrites. This causes concern since nitrites and piperine may be consumed simultaneously. Risk might increase with high-dose piperine supplementation. In another study, piperine appeared to enhance the bioavailability of aflatoxin B1 in rat tissues. And in yet another study, piperine was found to be cytotoxic to cultured brain neurons. Piperine was said to be non-mutagenic, however, in a study examining effects of the substance on the germ cells of Swiss albino mice.
In a recent study utilizing albino rats, piperine, given at doses of 5 and 10 mg/kg body weight for 30 days, resulted (at the 10-mg/kg dose level) in significant reduction in the weights of testes and accessory sex organs as well as severe damage to seminiferous tubules. The 5-mg/kg dose resulted in partial degeneration of germ cells.

Decreased mating performance, decreased fertility and anti-implantation activity, along with some other adverse reproductive events, were observed in mice given very high doses of piperine.

**Contraindications, Precautions, Adverse Reactions**

Piperine is contraindicated for those who are hypersensitive to any component of a piperine-containing preparation. Pregnant women and nursing mothers should avoid piperine supplementation. Piperine at doses generally higher than 15 mg daily may affect the metabolism of a wide range of drugs and xenobiotics. In some cases, doses lower than 15 mg daily may affect the metabolism of these substances. Those using the drugs listed in interactions should exercise caution in the use of piperine supplements.

Piperine may form mutagenic and possibly carcinogenic substances with nitrites. Those who eat processed food containing nitrites and nitrates as food preservatives should exercise caution in the use of piperine supplements.

**Adverse Reactions**

The typical dose of piperine in nutritional formulas is 5 milligrams, and doses of 15 milligrams daily are rarely exceeded. No adverse reactions have been reported with these doses. Piperine, if exposed to the tongue, is tasteless at first but leaves a burning aftertaste.

**Interactions**

Piperine, usually at a dose of 20 mg or greater, has been shown to inhibit the metabolism of the following drugs: propanolol, theophylline, phenytoin, sulfadiazene, rifampicin, isoniazid, ethambutol, pyrazinamide and dapsone. Piperine is a nonspecific inhibitor of drugs and xenobiotics. Most drugs metabolized via cytochrome P450 enzymes would likely be affected by piperine.

**Nutritional Supplements**

Piperine at a dose of 5 mg daily has been found to enhance the absorption of beta-carotene and coenzyme Q10. At a dose of 20 mg daily, it has been found to enhance
the absorption of curcumin. Piperine may also enhance the absorption of vitamin B₆, vitamin C and the mineral selenium in the form of L-selenomethionine.

Food

Piperine may enhance the absorption of beta-carotene, vitamin B₆, Vitamin C and L-selenomethionine found in certain foods.

Dosage and administration

Piperine is available in stand-alone supplements and in combination products. A typical dose is 5 mg daily. Doses higher than 15 mg daily should be avoided.

Piper longum linn differs little in its medicinal values from P. nigrum as it is less aromatic and more acrid. It is widely used in Ayurvedic and Unani systems of medicine particularly for diseases of respiratory tract. The dry spikes of female types are used in the ayurvedic preparations like Pipalarishta, Pipplayasava, Panchakola, Pippalayadiluha and Lavanabhaskar churnam. It is the major constituent of an ayurvedic preparation, 'Triaktu' which is prescribed routinely for a variety of diseases. The root is used for bronchitis, stomachache, diseases of spleen and tumours. It improves appetite also. The infusion of root is prescribed after parturition to induce the expulsion of placenta.

Long pepper contains the alkaloid piperine (about 6%), which is slightly higher than that in black pepper. Piperine has diverse pharmacological activities including nerve depressant and antagonistic effect on electro-shock and chemo-shock seizures as well as muscular incoordination. Thippali, as it is popularly known, also contains one per cent essential oil, which exhibits antibacterial activities.

Piperine, an alkaloid compound found in black pepper, stimulates the body's natural ability to generate heat and assists in the absorption of selenium and β-carotene. Piperine has been used to treat athletes and elderly people whose ability to absorb nutrients through their intestinal lining has been impaired.

Piperine, an alkaloid constituent of Piper longum linn, was evaluated for its anti-inflammatory activity in rats. Different acute and chronic experimental models like carrageenin-induced rat paw edema, cotton pellet granuloma, and croton oil-induced granuloma pouch, were employed. Piperine acted significantly on early acute changes in inflammatory processes and chronic granulative changes. It also acted partially through stimulation of pituitary adrenal axis.

In another study, piperine, an active alkaloidal constituent of Piper longum linn was evaluated for its anti-hepatotoxic potential in order to validate its use in traditional therapeutic formulations. The alkaloid exerted a significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both in
vitro and in vivo lipid peroxidation and by reducing the depletion of glutathione and total thiols.

Leaves and Stem contain: Beta- sitosterol, Nyctanthic acid, Iridoid, Glycerides of Lignocetric, Linocetric etc. Leaf extract is good in dandruff. Leaf extract is given in worms. Bark of the plant is good in cough and other respiratory diseases. Seeds crushed in water are applied on bald head. It romotes regeneration of hair. 5 g bark powder added in betel leaf (Pan) is given 3-4 times a day to patients of Asthma. In severe fever, leaf extract mixed with ginger extract and honey is said to be good.

Bark of the plant is useful in curing sciatica. Extract of the bark is prepared and applied over the painful parts. Lukewarm leaf extract of the plant mixed Karpur relieves the pain. Decoction of leaf mixed with Ginger and Garlic is applied in sciatica.

2.3 TRADITIONAL AYURVEDIC USES OF PIPER LONGUM LINN

- Pippali is certainly one of the most widely used of all Ayurvedic herbs. It is one of the best herbs for enhancing digestion, assimilation and metabolism of the foods we eat. It is also highly prized for its ability to enhance assimilation and potency of herbs in a synergistic formula (this is called the Yogavahi effect).

- The Ayurvedic texts list Pippali as one of the most powerful Rasayana herbs, meaning it is a longevity enhancer. It also cleans the shrotas that transport nutrients and remove wastes, so it is considered important for purification. It balances two of the three laws of nature at work in the mind and body (Vata and Kapha). It also soothes the nerves to improve the quality of sleep at night.

- Pippali enhances all 13 of the metabolic processes (Agnis) that create the 7 categories of bodily tissues (Dhatus).

- Along with Black Pepper and Ginger, Pippali is part of the famous digestive formula known as Trikatu (Three Spices).

Abana (Heart Care), Bonnisan, Geriforte (GeriCare / StressCare), Digyton, Geriforte Aqua, Geriforte Vet, Chyavanaprasha. All these formulations have been indicated for their treatment in cardiac related problems.

Various Formulations of Piper longum linn

Trikatu – Ayurvedic formulation containing Piper longum linn:

Black pepper and long pepper have been used in Ayurvedic medicine for the treatment of various diseases. One such preparation is known by the Sanskrit name 'Trikatu' & Consist of black pepper, long pepper & ginger. It is thought that piperine is one of the major bioactive substances of these Ayurvedic remedies. Black pepper
has also been used in traditional Chinese medicine to treat seizure disorders. A derivative of piperine, antiepileptirine, has also been used in China to treat seizure disorders. Some recent research suggests that piperine may enhance the bioavailability of some drugs and nutritional substances.

Trikatu is a traditional Ayurvedic herbal formulation consisting of three herbs in equal amounts, Black pepper (*Piper nigrum*), Long pepper (*Piper longum linn*) and Ginger (*Zingiber officinale*).

'Trikatu' is a sanskrit word meaning 'three acrids', referring to the pungent qualities of the three ingredient herbs. The use in Ayurveda of these three herbs is documented in the ancient Ayurvedic materia medica dating back several thousands of years. In Ayurveda, the ingredients of Trikatu are important components of numerous formulations used for a wide range of disorders [37-38].

According to Ayurvedic philosophy, disease results from imbalance between the three humours of the body, *kapha, vata* and *pitta*. It has been suggested that the acrid or pungent ingredients of Trikatu act to restore the balance of these humours, a hypothesis that offered an explanation for the widespread application of these herbs. In terms of Ayurveda, Trikatu is also described as rejuvenating digestive fire (*agni*) and burning away toxic build-up in the digestive tract (*ama*), thus facilitating proper digestion, assimilation and metabolism as well as elimination.

**Composition and constituents**

As mentioned above, Trikatu consists of equal parts Black pepper (*Piper nigrum* dried fruit), Long pepper (*P. longum* dried fruit) and Ginger (*Zingiber officinale* dried rhizome). Both Black and Long pepper contain as their major active constituent the alkaloid piperine, which is chiefly responsible for the pungency of these peppers.

Black pepper contains 5-9% of the alkaloids piperine and piperettine and 1-2.5% of volatile oil, the major constituents of which are alpha- and beta-pinene, limonene and phellandrene [39-40]. In one study, the essential oil of Black pepper was found to comprise 33.7% beta-caryophyllene [41].

Long pepper was found to contain about 1.25% piperine as well as about 1% volatile oil, the major constituents of which were beta-caryophyllene (17%), pentadecane (17.8%) and beta-bisabolene [14] (11.16%).

Long pepper also contains an amide, which has demonstrated coronary vasorelaxant activity. The major pungent compound in dried Ginger rhizome is (6)-shogaol, the dehydration product of (6)-gingerol, which is the primary pungent compound in fresh Ginger. Shogaol is more pungent than gingerol.[42] Ginger also contains a volatile oil, which shows considerable variation depending on geographical origin. Ginger from India typically yields a volatile oil containing high levels of zingiberene and ar-curcumene [43-44].
Indications for Trikatu

Trikatu works primarily in the digestive tract, where it assists proper digestive function through stimulation of digestive enzyme activity. Trikatu also acts on the respiratory system. Trikatu would not normally be used by itself, but incorporated into formulations with other herbs.

Indications for Trikatu include:

- Indigestion
- Flatulence
- Colic
- Weak digestion.
- Poor absorption
- As an adjuvant to increase the bioavailability of other herbs
- Intestinal infection
- Common cold
- Cough

Contraindications and cautions

Trikatu is for professional use only. Because of its pungency, Trikatu it not suitable for children. Since Trikatu may affect the bioavailability of other compounds, it should not be administered to people who are taking prescription drugs, without close monitoring by the prescribing practitioner.

Similarly, Trikatu may affect (most likely increase) the bioavailability of other therapeutic substances, including herbal medicines and nutritional supplements, and the dose of these may need to be adjusted accordingly.

Chitrakadi Tablet

Indications

Chitraka is the name of fire. This formulation is best for improper or lake of digestive fire. It works on indigestion, lake of appetite, bulimia, abdominal gas and remove Ama dosha's which are due to lake of fire. Total strength is 250 mg.

Chitrakmoor (Plumbago zeylanica), Pippalimool (roots of Piper longum linn), Yavakshar Sajjikshar, Lavang, Hingu, Ajamoda, Chavya (Piper chaba hunter), Sunthi (Zingiber officinale), Marich (Piper nigrum).

Doses: 1 tablet for 2 to 3 times daily
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Bioperine (Black pepper extract)
Bioperine® is a standardized extract obtained from black pepper, containing not less than 95% piperine. The product is a natural bioavailability enhancer for nutrients and is proven to improve the uptake and utilization of several nutritional supplements, including vitamins, antioxidants and minerals [45-46].

Cardana is a formulation of botanicals used in traditional Ayurvedic medicine to support the health of the heart and vasculature, including supporting the healthy supply of blood to the heart and supporting healthy blood pressure. These traditional uses are now supported by scientific evidence. Serving Size: 2 Tablets

Terminalia arjuna (25% Tannins) .............................................. 200mg
Cratagus oxyacantha (2% Vitexin) .............................................. 200mg
Coleus forskholli 4% (4% Forskohlin) ........................................... 200mg
Withania somnifera 3% (2.5% Withanolides: 0.1% Sitoindsides) ... 100mg
Boerhaavia diffusa (0.1% Akaloids) ........................................... 100mg
Piper longum (10% Piperine) ..................................................... 5mg
Other ingredients: tragacanth, microcrystalline cellulose, silicon dioxide.

Main Applications: Anti-Hypertensive, Cardiotonic, Oxygen sparing, Anti-Stress.

Aller-7
It is a combination of the following seven herbal extracts [47] Phyllanthus emblica (fruit), Terminalia chebula (fruit), Terminalia bellerica (fruit), Albizia lebbeck (bark), Zingiber officinale (root), Piper longum (fruit) and Piper nigrum (fruit).

Allergic rhinitis or hay fever is a common health problem for over 40 million Americans. The well-known, and unwelcome, symptoms include sneezing, runny and/or stuffed nose, itchy nose and eyes, and congested sinuses.

Reports indicating [48] use of piper fruits in malaria was also reported in 1983 symposium in Bombay, India entitled “Therapeutic Approaches to Malaria” sponsored by Ciba Geigy, Ltd., Long pepper fruits were given in an increasing dose from 3 to 30, starting with 3 and increasing daily by 3 fruits. Subsequently the dose was decreased from 30 to 3 fruits, by reducing 3 fruits daily. Long pepper was boiled in milk and water and drank once a day in the early morning. Drinking this decoction reportedly caused cessation of malarial parasite multiplication and regression of splenomegaly.

Nervshield is a potent herbal psychical supplement. It soothes and helps reduce anxiety by helping the brain to focus on reality. It is a safe multi-herbal formulation designed to help battle anxiety and its after effects.
Each capsule contains:

*Centella asiatica*, *Withania somnifera*, *Piper longum*, *Evolvulus asinoides*,
*Rauwolfia serpentina*, *Tinospora cordifolia*, *Hyocyamus niger*, *Glycyrrhiza glabra*,
*Piper nigrum*, *Embelia ribes*

**Triphala Guggulu**

**Indication:** Fistula, piles/hemorrhoids, edema, constipation and gout

**Presentation form:** Vati Tablet

**Description**

This formulation of herbs acts as a rejuvenator and alleviates the aggravated vata and related disorders. It is highly recommended in constipating condition that causes piles, fistula and edema. It also works as a laxative, an appetizer and blood purifier. All the herbs are collected, finely sorted and then ground into fine powder, which is then made into tablets. This powder is then made into tablets.

**Directions for use**

Twice daily preferably with lukewarm milk or water or as directed by the physician.

**Dosage**

2 - 4 tablet twice daily or as directed by the physician.

**Action**

The Indian gooseberry contains gallic acid, tannic acid and albumen and is useful for toning the intestine. Haritaki is one of the best herbs for balancing vata dosha. As a natural laxative and purgative, it removes undigested food and accumulated toxins from the gastrointestinal tract. Triphala acts as a tonifying blood cleanser and gentle laxative, highly prized for its ability to regulate the processes of digestion and elimination. Translated as "three fruits" it is composed of the dried fruits of amalaki, bibhitaki and haritaki. Triphala readily removes excess vata, pitta and kapha from the body, bringing balance and proper functioning to the system. Pippali (*Piper longum*) raises digestive fire and clears kapha. *Commiphora mukul* gum is the most effect herb used in the Ayurvedic treatment of arthritis. It has strong rejuvenating, purifying, medicinal and healing qualities.

**Ingredients:** *Terminalia chebula*, *Terminalia bellirica*, *Emblica officinali*, *Piper longum*, *Commiphora mukul*

**Pippali Tablets (Piper Longum)**

Used traditionally as a digestive, carminative (removes excess intestinal gas), sexual stimulant, general tonic, and mucolytic.
**Chonsath Prahari Pippal**

This classical ayurvedic formulation contains highly potentised pippali (*Piper longum*) that revitalizes all body systems, stimulates liver, improves general metabolism and raises immunity level. It's a potent cardiotonic, highly effective in allergic and chronic cough & cold and all types of respiratory disorders. It is available in 15 gm packing.

**RespiNova**

RespiNova is a clinically proven comprehensive cough remedy efficacious in all types of cough. RespiNova reduces the frequency and severity of cough, helps expel sputum through its mucolytic and expectorant effects, and thus relieves cough most reliably. RespiNova is free from sedation.

**Therapeutic category:** Cough formulation for adults.

**Indications:** Cough associated with LRTI/ URTI Bronchitis

Smokers cough Allergic cough.

**Clinical Trial:** An open clinical trial in patients with LRTI revealed significant reduction in severity & frequency of cough & sputum quantity

**Composition:** Each 5 ml contains

- *Yeshtimadhu (Glycerrhiza glabra)* ........................................ 48 mg
- *Vasa (Adhatoda vasica)* ..................................................... 26 mg
- *Tulasi Ocimum sanctum)* .................................................. 15 mg
- *Shunthi Zingiber officinale)* ............................................ 4.8 mg
- *Pippali(Piper longum)* ................................................... 9.8 mg
- *Pudina Mentha piperata* .................................................... 05 mg
- *Shirish Albezzia lebbeck)* ................................................ 15 mg
- *Haridra Curcuma longa)* .................................................. 10 mg
- *Pushkarmool Inula racemosa)* ........................................... 15 mg

**Dosage:** 2-4 teaspoonfuls thrice daily or as directed by the physician

**Safety:** No adverse effects are observed at recommended doses

**Available as:** Syrup 100 ml

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**Cardiac Activity and Formulation Evaluation of Isolated Constituents of *Piper longum* linn**
2.4 PRESENT SCENARIO OF THE PROPOSED WORK

Literature survey shows that no data is available on the pharmacological properties of different constituents of the *Piper longum linn*. The systematic investigation of the *Piper longum linn*, could lead to a development of better drug molecules for the treatment of different diseases.

Combined formulations of *Piper longum linn* extracts along with other plants shows good therapeutic activity against heart diseases, however literature survey shows that very less data is available on the cardiovascular properties of the constituents of the *Piper longum linn* hence proper qualification and quantitative analysis of the plant is very essential for exploring the cardiac activity of the constituents.

Cardiovascular diseases have posed a serious challenge in front of us. Globally millions of people are suffering from heart diseases. Hence, there is a urgent need to find out the new plant derived natural drug molecules to fight against the heart diseases. The safety and efficacy of the plant *Piper longum linn* and their species have already been proved by their use in the traditional system of medicine and is also used as a spicy food material.

The cardiac studies on laboratory animals will be useful for exploring the exact role of plant constituents.

**Experimental work has been divided in following steps:**

1) Isolation
2) Characterization
3) Pharmacological Studies
4) Formulation and Evaluation

**Isolation**

**Method of extraction:**

Piperine can be isolated from the oleoresin of *P. nigrum* or *Piper longum linn*. The powdered fruits of the plant are extracted with dichloromethane at room temperature with stirring for 12 hours. The extract is filtered, concentrated in vacuum, and then the residue is purified on an alumina column. Pure piperine can also be obtained by crystallization from ethanol, which may be required for food and/or medicinal usages. Piperine is obtained directly from the crude 3 residue in lesser amounts by extraction in alcohol, filtration and successive crystallization.

Piperine may facilitate nutrient absorption by alleviating inflammatory conditions at the site of absorption. The mechanisms behind the beneficial action of piperine as one of the principal ingredients of numerous digestive formulas employed by Ayurveda needs to be further investigated. Particular emphasis needs to be placed on
the traditional sense of restoring gastrointestinal function as means of preventing
disease and improving overall nutrition. Black pepper and long pepper are thus
potentially useful herbs in the management of a variety of respiratory and
gastrointestinal problems. Future research on pepper may well retrace the origin and
evolution of the properties which attracted attention to pepper in ancient times. Pliny
commented some 2,000 years ago: "it is quite surprising that the use of pepper has
come so much in fashion, its only desirable quality being a certain pungency; and yet
it is for this that we import it all the way from India!" This pungency of pepper is
now understood to be a byproduct of the biological properties of piperine, which can
apparently regulate neurohormones, thereby increasing thermogenesis, or the
production of heat by the body [49]. Scientific research has now revealed that the
"hot" pepper taste is due to the production of heat energy.

Column chromatography was used for the isolation of different constituents of plants
[50].

Literature survey shows that very few methods are available for the separation or
isolation of different constituents form the plant *Piper longum linn*. The review of
methods which are used to isolate different constituents form the different parts of
the plant *Piper longum linn* in some of the methods and the activity of different
solvent extracts is also mentioned as:

Wu, Shihua [51] et al, (2004) reported preparative isolation and purification of
amides from the fruits of *Piper longum linn* by upright counter-current
chromatography and reversed-phase liquid chromatography. They have reported to
carry out the extraction process by using light petroleum ether. Counter-current
chromatography (CCC) technique was used for the isolation of the plant constituents.

Park, Byeoung Soo [52] et al, (2003) reported isolation of Piperoctadecalidine, a
piperidine alkaloid, of the hexane fraction from the fruits of *Piper longum linn* using
chromatographic techniques. The compounds were found to show fungicidal activity.

Lee, Sung Eun [53] et al, (2002) isolated the alkaloidal constituents like,
pipeclongumine, piperine, pipemonaline, and piperoctadecalidine, from *Piper
longum linn*, which were found to inhibit the biosynthesis of aflatoxin B1 (AFB1) in
Aspergillus flavus WRRC 3-90-42-12. Piperlongumine was the most active among
the compounds tested. The three other piperidine alkaloids, piperine, pipemonaline,
and piperoctadecalidine, also inhibited the biosynthesis of AFB1.

Bajad, Sunil [54] et al, (2002) reported a High-Performance Liquid Chromatography
(HPLC) method for the analysis of piperine in rat plasma. Analysis was performed
using a Symmetry(R) C18 column (250 x 4.6 mm) by isocratic elution with 25 mM
KH2PO4 (pH 4.5)-acetonitrile (35:65) and UV detection at 340 nm.

linn* fruit-derived materials against the fourth-instar larvae of Aedes aegypti was
reported. A crude methanol extract of *P. longum* fruits was found to be active against
the larvae, and the hexane fraction of the methanol extract showed a strong larvicidal
activity of 100% mortality. The biologically active component of *P. longum* fruits was characterized as pipemonaline by spectroscopic analyses. The toxicity of pipemonaline is comparable to that of pirimiphos-methyl as a mosquito larvicide.

Park, Byeong Soo [56] et al, (2002) reported isolation of pipemonaline and piperoctadecalidine, from the fruits of the plant *Piper longum* linn.

Lee, Sung Eun [57] et al, (2001) also reported fungicidal activity of pipemonaline, a piperidine alkaloid derived from long pepper, *Piper longum* linn., against phytopathogenic fungi.


Madhusudhan, P. [59] et al, (2001) reported tetrahydropiperine, the first natural aryl pentanamide from *Piper longum linn* fruits.

Lee, Sung Eun [60] et al, (2000) reported a methanol extract of *Piper longum linn* fruit was found to be active against mosquito larvae of *Culex pipiens pallens* Latha, C [61] et al, (1999) shows that Petroleum ether extract of *Piper longum linn* shows promising activity as potential biopesticides.


Shankaracharya, N. B. [14] et al, (1997) reported that long pepper contained about 1% volatile oil, 1.25% piperine and 40% starch. As compared to black pepper, long pepper was poor in essential oil and piperine and the volatile oil was dextrorotatory, while that of black pepper was levorotatory. The GC-MS analysis of the essential oil showed the presence of 48 components, out of which 44 were identified for the first time. The three major components of the oil, identified were beta-caryophyllene (17%), pentadecane (17.8%) and beta-bisabolene (11.16%).

Das,Biswanath [63] et al, (1996) have reported alkamides of *Piper longum linn*.

Zhang-Ke [64] et al, (1996) reported a new dimer of amide from *Piper longum linn*, the dimer of desmethoxypiplartine, together with four known compounds, (Z)-12-octadecenic-alpha-glycerol monoester, piperine, beta-sitosterol, daucosterol, were isolated from *Piper longum linn*.

Desai, S. J. [65] et al, (1989) reported a new aristolactam, 10-amino-2, 3, 4-trimethoxyphenanthrene-1-carboxylic acid lactam has been isolated from *P. longum*.

Desai, S. J. [66] et al, (1988) reported nine alkaloids were isolated from the cold ethanol extract of *Piper longum linn* roots, of which six known compounds were identified as cephadrione B, cephadrione A, cepharanone B, aristolactam AII, norcephadrione B, and 2-hydroxy-1-methoxy-4H-dibenzo[de,g]quinoline-4,5(6H)-dione. The three new alkaloids were characterized as 10-amino-4-hydroxy-3-methoxyphenanthrene-1-carboxylic acid lactam [piperolactam A], 10-amino-4-hydroxy-2, 3-dimethoxyphenanthrene-1-carboxylic acid lactam [piperolactam B] and
2-hydroxy-1-methoxy-6-methyl-4H-dibenzo [de, g] quinoline-4, 5(6H)-dione [piperadione].

Shoji, N. [42] et al, (1986) reported an amide (dehydropipernonaline) that has coronary vasorelaxant activity was isolated from the fruit of *Piper longum linn*. This substance was characterized on the basis of spectroscopic data.

Banerjee, T. [67] et al, (1986) reported the crystal and molecular structure of N-3 4 5 trimethoxycinnamoyl-delta-3-piperidin-2-one an Amide Alkaloid Piperlongumine Isolated from *Piper longum linn*.


Chandhoke N [69] et al, (1978) reported for post-coital antifertility activity of *Piper longum linn* in rats. Activity was most marked in petroleum ether extract of *P. longum*. Among the natural amides screened, pipeline, piplaritine, seven semisynthetic analogs of piperine, Pyrrolidine amide of piperic acid and pipronyl acid showed some activity.

Kholkute, S. D. [05] et al, (1979) reported anti fertility effects of the fruits of *Piper longum linn* in female rats. The benzene extract exhibited 57% inhibitory activity while the chloroform extract showed 50% activity. The Petroleum ether and the methanol extracts as well as the powdered fruits revealed only marginal antifertility activity. The most potent extract of *P. longum* when mixed with the methanol extract of *Embelia ribes* berries and administered to female rats inhibited pregnancy in 80% of the animals.

Dutta, C. P. [70] et al, (1977) reported isolation of piperlongumine, piperlonguminine, piperine, sesamin, 2 minor constituents and methyl 3,4,5-trimethoxycinnamate from the roots of *Piper longum linn*.

Chattergee, A. [71] et al., (1967) reported synthesis of alkaloids of *Piper longum linn*.

*Different constituents [72-73] of Piper Species are as follows:*
Cardiac Activity and Formulation Evaluation of Isolated Constituents of Piper longum linn
The above figure gives examples of major classes of compounds found in *Piper* spp. Stereochemistry is depicted in all cases for which it is known. **Amides:** cepharadione A (1) and piperine (2); **Propenylphenol:** safrole (3); **Lignan:** sesamin (4); **Neolignan:** kadsurin A (5); **Terpenes:** transphytol (6), terpinolene (8); **Kawapyrone:** methysticin (7); **Dihydrochalcone:** asebogenin (9); **Flavone:** 7,4-dimethoxy-5,3-dihydroxyflavone (10); **Other:** dopamine (11).

Other amides that have been studied in an ecological context include pipericide, piplartine, 4-desmethyl piplartine, and cenocladamide; the latter two compounds appear to be unique to *P. cenocladum*.

![Amides from *Piper cenocladum*: piplartine (12), 4-desmethylpiplartine (13), and cenocladamide (14).](image)

Hexane extract of dried fruits of *P. longum* has been reported to yield a new alkamidine, isodihydropiperlonguminine (16). Two naturally occurring phenylpropanoids, 3-(3,4,5-trimethoxyphenyl)-propanoic acid methyl ester (17), methyl dihydroferulate (18), pipataline (19) and piperittine (20) were also reported to be isolated.

![Piperlonguminine (15)](image)
Chapter 2 Literature Survey

Isodihydropiperlonguminine (16)

Phenylpropanoids, 3-(3,4,5-trimethoxyphenyl)-propanoic acid methyl ester (17)
(R = -OMe)

Methyl dihydroferulate (18)

Pipataline (19)
**Pharmacological Studies**

**Acute toxicity studies**

Acute toxicity studies have to be carried out to find out the safe dose of drug molecule so that it can be used as benchmark for further studies. These studies will be carried out as per the guidelines of OECD (Organization for economic cooperation and development Guideline No.425) for the testing of chemicals.

**Isolated Frog Heart Preparations**

Extracts of the plant *Piper longum linn* has been indicated in the heart diseases. Different part extracts of *Piper longum linn* have been used as an active extract in various ayurvedic formulations and preparations, due to the beneficial role of pharmacological actions of *Piper longum linn* in cardiac diseases, however systematic investigation of the plant extract and its constituents has not been done to explore the exact role of the plant and its constituents in the heart diseases. Isolation of the different constituents from the plant can be subjected for evaluation of their activity on hearts. Guede-Guina F et al, (1997) [74] has mentioned the use of plant extracts and its actions on isolated frog heart preparations. Roots of the plant *Piper longum linn* can be used for cardiac studies of different constituents on isolated frog heart. For screening of different constituents of *Piper longum linn*, a well established isolated frog heart experiment can be selected. Isolated frog heart experiment is one of the prime and unique experiment which can be used as marker experiment to study the preliminary actions of the plant extract and different constituents. This experiment is very reproducible, economic and can be used as a cardiac activity marker in the initial phases of evaluation of the activity of any drug substances. The isolated constituents can be thus subjected for the cardiac activity on the isolated frog heart as per the reported methods.

**Enzymatic activity of Piper longum linn and its constituents:**

The isolated constituents can also be subjected to study their protective effects in case of myocardial ischemia.

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**Piperittine (20)**
Myocardial infarction

Insufficient blood supply to the myocardium can result in myocardial ischemia, injury or infarction, or all three. Atherosclerosis of the larger coronary arteries is the most common anatomic condition to diminish coronary blood flow. The branches of coronary arteries arising from the aortic root are distributed on the epicardial surface of the heart. These in turn provide intramural branches that supply the cardiac muscle. Myocardial ischemia generally appears first and is more extensive in the sub-endocardial region since these deeper myocardial layers are farthest from the blood supply, with greater intramural tension and need for oxygen.

Subendocardial ischemia

Ischemia in this area prolongs local recovery time. Since repolarization normally proceeds in an epicardial-to-endocardial direction, delayed recovery in the subendocardial region due to ischemia does not reverse the direction of repolarization but merely lengthens it. This generally results in a prolonged QT interval or increased amplitude of the T wave or both as recorded by the electrodes overlying the subendocardial ischemic region.

Subepicardial or transmural ischemia

Transmural ischemia is said to exist when ischemia extends subepicardially. This process has a more visible effect on recovery of subepicardial cells compared with subendocardial cells. Recovery is more delayed in the subepicardial layers, and the subendocardial muscle fibers seem to recover first. Repolarization is endocardial-to-epicardial, resulting in inversion of the T waves in leads overlying the ischemic regions.

Electrocardiographic changes in Myocardial ischemia

Injury to the myocardial cells results when the ischemic process is more severe. Subendocardial injury on a surface ECG is manifested by ST segment depression, and subepicardial or transmural injury is manifested as ST segment elevation.

During acute myocardial infarction, the central area of necrosis is generally surrounded by an area of injury, which in turn is surrounded by an area of ischemia. Thus, various stages of myocardial damage can coexist. The distinction between ischemia and necrosis is whether the phenomenon is reversible. Transient myocardial ischemia that produces T wave, and sometimes ST segment abnormalities, can be reversible without producing permanent damage and is not accompanied by serum enzyme elevation. Two types of myocardial infarction can be observed electrocardiographically:
• Q wave infarction, which is diagnosed by the presence of pathological Q waves and is also called transmural infarction. However, transmural infarction is not always present, hence, the term Q-wave infarction may be preferable for ECG description.

• Non-Q wave infarction, which is diagnosed in the presence of ST depression and T wave abnormalities.

Elevation of serum enzymes is expected in both types of infarction. In the absence of enzyme elevation, ST and T wave abnormalities are interpreted as due to injury or ischemia rather than infarction.

**Animal models and methods for inducing myocardial ischaemia**

The complete ligation of the left anterior descending coronary artery, LAD in Sprague Dawley rats results in myocardial infarction of variable size. After 3-6 weeks overt heart failure occurs in a subset of animals with a myocardial damage of 40 to 50% of the left ventricular circumference. These rats are well characterized by cardiac, haemodynamic and neurohumoral alterations such as increased ventricular filling pressures, left ventricular LV dilatation, decreased cardiac index, myocardial necrosis and apoptosis, activation of various cardiac and circulating peptide systems e.g. natriuretic peptides, renin angiotensin system. [75-77]

Due to its evident clinical relevance and the relatively easy technique, myocardial infarction in the rat is a widely used small animal model of heart failure. However, there are some restrictions, which should take into consideration. There is a high initial mortality up to 50% due to surgical procedure and or complications of myocardial infarction. Furthermore, the infarction size varies greatly between 5 and 59% of the left ventricular circumference depending on the anatomical variability of the pattern of coronary arteries in Sprague-Dawley or Wistar Kyoto rats. Only rats with myocardial damage greater than 40-50% will develop signs of heart failure such as ascites or respiratory distress after 3-6 weeks [78]. Muliniari et al. [79] reported that even a subgroup of rats with large myocardial infarction with damage greater than 66% did not develop significant alterations in cardiac output, blood pressure or peripheral resistance. In another study rats were investigated at 4, 8 and 16 weeks after coronary artery ligation. Interestingly, only rats of the 16-week group consistently exhibit haemodynamic and clinical signs suggestive of heart failure [80]. At 4 or 8 weeks only some of these abnormalities were present. In conclusion, these findings suggest that this model might be more valuable for studies in chronic heart failure if the experiments are performed after a longer interval following coronary ligation. Furthermore, a large number of rats is needed due to the high mortality and marked variability in infarct size and cardiac dysfunction.

Recently, it has been suggested that the branching pattern of coronary arteries in Lewis-inbred rats is more consistent than in other strains [81]. Ligation of the LAD in Lewis-inbred rats may produce a uniformly large infarct with a lower mortality.
rate than in comparison with Sprague-Dawley rats. It should be noted that clinical signs of chronic heart failure and neurohumoral alterations have not been evaluated in this study. Therefore, further studies are needed for a more detailed description of this promising model.

Ischaemic heart disease in humans results from non-occlusive coronary artery constriction. In the rat, this situation can be imitated by reduction of the inner luminal diameter of the left coronary artery of approximately 60% by ligation of a probe held in contact with the vessel and then removal of the probe [82-83].

The reduction in coronary blood flow leads to a predictable progression in impairment of LV performance and heart failure. This model seems to allow a more moderate development of ischaemic heart failure in comparison with complete occlusion and the overall mortality rate is approximately 43%. However, it also lacks the aspect of atherosclerosis, which is the major cause of the chronic narrowing of human coronary arteries. Embolization of smaller intramycardial vessels by plastic microspheres produces a decrease in stroke volume and cardiac index whereas LV filling pressure and total peripheral resistance increase [84]. This model will have similar advantages and disadvantages to the model of ligation of the coronary artery as mentioned above.

In the present study in order to study to understand the role of plant extract and its constituents, it was decided to study the effects of the plant on enzymatic levels in rat’s heart.

Myocardial injury (MI) commonly occurs following ischemia and reperfusion [85]. Myocardial ischemia and reperfusion are characterized on the one hand by increased generation of reactive oxygen species and by a decrease in endogenous anti-oxidant mechanisms on the other [86]. Isoproterenol (ISO) is a β-adrenergic agonist and produces acute myocardial necrosis in high dosages [87–89]. Some of the mechanisms proposed to explain the ISO-induced injury to myocardial cells include hypoxia, calcium overload and excessive production of free radicals resulting from oxidative metabolism of catecholamines [90–92].

Catecholamines are important regulators of myocardial contractility and metabolism. However, it has been known for a long time that excess catecholamines are responsible for cellular damage, observed in clinical conditions such as transient myocardial ischemia, angina, acute coronary insufficiency, and subendocardial infarct[93]. Catecholamine-induced myocardial injury is a classical example of “stress cardiomyopathy” [94]. This term is also used to denote the cause of sudden unexplained death, elicited by extreme stressful life circumstances [95]. Excessive endogenous release or exogenous administration of catecholamines depletes the energy reserve of cardiac muscle cells. This leads to complex biochemical and structural changes that cause irreversible cellular damage, which is a prelude to necrosis [96].

Cardiac Activity and Formulation Evaluation of Isolated Constituents of Piper longum linn
Administration of large amounts of catecholamines, particularly isoproterenol, to experimental animals constitutes a rapid and reproducible means of provoking myocardial ischemia [97]. Myocardial infarction occurs when myocardial ischemia exceeds a critical threshold level for an extended time period and results in irreversible myocardial cell damage or death [98]. It appears that, although the pathogenesis of catecholamine-induced myocardial necrosis is multifactorial, oxidative stress plays a major role in it [99]. Since catecholamines rapidly undergo oxidation, it has been suggested that the oxidative products of catecholamines are also responsible for myocardial changes [100].

A better understanding of the process involved in myocardial ischemia has stimulated the search for new drugs that could limit the myocardial injury. The major abnormalities noticed in myocardial ischemia are lipidemia, peroxidation, and loss of plasma membrane integrity. Drugs with multiple mechanisms of protective actions, including antioxidant properties, may be one way forward in minimizing cell injury on myocardial ischemia. Given the potential importance of antioxidant therapy against catecholamine-mediated myocardial injury in humans, a number of experimental studies have sought to determine the effects of dietary antioxidants on myocardial cell protection against catecholamine-induced toxicity. Medicinal plants play a key role in human health care nowadays. While in acute conditions, such as myocardial necrosis, the modern medicine is the only answer, it is increasingly being understood that the traditional Indian medicine does have a remedy for these acute disorders.

The diagnostic marker enzymes of myocardial infarction are creatine kinase (CK), lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AST). Rats administered with isoproterenol shows a significant decrease in activities of enzymes such as CK, LDH, AST and ALT in the heart with subsequent increase in their activities in serum when compared to normal.

**Protective antioxidant effect or activity of Plant material in myocardial ischemia**

A significant increase in the levels of lipid peroxides in serum and heart on isoproterenol administration indicates enhanced lipid peroxidation by free radicals. Due to increased lipid peroxidation, glutathione levels can be significantly lowers in blood and heart of animals. Decreased glutathione level may be due to its increased utilisation in protecting SH groups containing proteins from the action of free radicals. Glutathione participates directly in the destruction of hydrogen peroxide and also promotes the formation of reduced form of ascorbate, which has high antioxidant activity.

Drugs exerting protective effects on heart may act by their antioxidant activity by lowering the lipid peroxides and maintaining the normal levels of glutathione. [101].
**Histopathological changes in heart**

To understand the effect of drugs on tissues at cellular level, histopathological changes is the marker experiment. The normal heart tissues can be stained and careful monitoring of different slides of sections of heart tissue gives an idea about the different cellular changes that occur in tissue. In case of myocardial ischemia myofibrillar degeneration which is associated with infiltration of neutrophil granulocyte and interstitial edema are observed. The differences in tissues give idea about the extent of necrosis and damage to the heart tissue[102]

**Antibacterial studies**

Plant and plant derived products have always found to play vital role in the treatment of infections cause by bacteria. Bacterial resistance to drugs, cost of drugs and toxicity of available drugs necessitates to search for new drugs which can treat bacterial infection completely. Many of the bacterial stains forund to be pathogenic some of them are discussed as follows:

I. **E. Coli:**

*E. coli* O157:H7 infection often causes severe bloody diarrhea and abdominal cramps, sometimes the infection causes nonbloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2%-7% of infections lead to this complication. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* O157:H7.

II. **Pseudomonas** species

For an opportunistic pathogen such as *Pseudomonas aeruginosa*, the disease process begins with some alteration or circumvention of normal host defenses. The pathogenesis of *Pseudomonas* infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium. Multiple and diverse determinants of virulence are expected in the wide range of diseases caused, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis.

Most *Pseudomonas* infections are both invasive and toxinogenic. The ultimate *Pseudomonas* infection may be seen as composed of three distinct stages: (1) bacterial attachment and colonization; (2) local invasion; (3) disseminated systemic disease. However, the disease process may stop at any stage. Particular bacterial determinants of virulence mediate each of these stages and are ultimately responsible for the characteristic syndromes that accompany the disease.
Endocarditis Pseudomonas aeruginosa infects heart valves of IV drug users and prosthetic heart valves. The organism establishes itself on the endocardium by direct invasion from the blood stream.

III. Shigellosis

Shigellosis is an infectious disease caused by a group of bacteria called Shigella. Most who are infected with Shigella develop diarrhea, fever, and stomach cramps starting a day or two after they are exposed to the bacterium. The diarrhea is often bloody. Shigellosis usually resolves in 5 to 7 days. In some persons, especially young children and the elderly, the diarrhea can be so severe that the patient needs to be hospitalized. A severe infection with high fever may also be associated with seizures in children less than 2 years old. Some persons who are infected may have no symptoms at all, but may still pass the Shigella bacteria to others.

IV. Salmonella

Salmonella species can cause diseases ranging from gastroenteritis to typhoid fever, and can be transmitted through the ingestion of contaminated food or water. Upon entering the host cell there is an increase in the levels of intracellular free calcium as well as a rearrangement of the cell cytoplasm. Salmonella also ruffles the membrane, which appears to be an important part in the entry process. The most common Salmonella infections (generally by Salmonella enterica serovar Enteritidis) generally cause diarrhea, fever, or abdominal cramps. Salmonellosis can usually resolve itself without treatment 5-7 days after infection unless the host becomes severely dehydrated or if the infection spreads. While it generally can be treated with ampicillin, gentamicin, trimethoprim/sulfamethoxazole, or ciprofloxacin, some of the bacteria have become resistant to certain antibiotics as a result of using antibiotics to promote the growth of feed animals. Although most people with this type of Salmonella infections usually recover completely, a small amount of people develop Reiter's syndrome which causes pains in their joints, irritation of their eyes, and painful urination. This syndrome can last for months, years, or can even lead to chronic arthritis.

V. Pathogenesis of S. aureus infections

Staphylococcus aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. S. aureus is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling
medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the blood stream.

*S. aureus* expresses many potential virulence factors like

- Surface proteins that promote colonization of host tissues
- Invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase)
- Surface factors that inhibit phagocytic engulfment (capsule, Protein A)
- Biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production)
- Immunological disguises (Protein A, coagulase, clotting factor)
- Membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin)
- Exotoxins that damage host tissues or otherwise provoke symptoms of disease (SEA, TSST, ET)
- Inherent and acquired resistance to antimicrobial agents.

VI. *Klebsiella pneumoniae* and *Klebsiella oxytoca*

These are both opportunistic pathogens found in the environment and in mammalian mucosal surfaces; they are commonly passed by hands of hospital personnel. Common sites for nosocomial *Klebsiella* infections include the urinary tract, lower respiratory tract, biliary tract, and surgical wound sites. Clinical syndromes caused by this bacteria include pneumonia, bacteremia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, and meningitis. Infection in the lungs, called pneumonia, leads to necrosis, inflammation, and hemorrhage in the lung tissue, which produces a thick, bloody, mucoid sputum called currant jelly sputum. People at high risk to get this are middle-aged to older men with alcoholism, diabetes, or chronic bronchopulmonary disease. Two rarer infections caused by *Klebsiella* bacteria are rhinoscleroma, a "chronic inflammatory process involving the nasopharynx," and ozena, a "chronic atrophic rhinitis characterized by necrosis of nasal mucosa and mucopurulent nasal discharge" (Emedicine).

VII. *B. cereus*

Infections in humans and animals leads to watery diarrhea, cramps, abdominal pain, nausea but not vomiting, wound infections, septicemia, mastitis and meningitis.
VIII. *Serratia marcescens*

It is considered a harmful human pathogen which has been known to cause urinary tract infections, wound infections, and pneumonia. *Serratia* bacteria also have many antibiotic resistance properties which may become important if the incidence of *Serratia* infections dramatically increases. *Serratia* can be distinguished from other genera belonging to Enterobacteriaceae by its production of three special enzymes: DNase, lipase, and gelatinase.

It is well documented from ancient times that the active principles from plant origin have been used as medicines for various diseases and microbial infections. These active principles from plant origin have provided numerous crucial molecules in the search of new drug medicines [103] The search of natural products has revolutionized the drug discovery programme. Many plant derived molecules have shown a promising effect in therapeutics. The diverse behavior of bacteria has always presented challenge in the treatment of their infections. Very few antibiotics are effective against *Pseudomonas* including floroquinolones, gentamycin and imipenem. Even these antibiotics are not effective against all strains. The children (below 5 years age) who are susceptible to *E. coli* infection shows the symptoms of hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidney fails, about 2-7% cases showed these type of complications [104]. The resistance of bacteria against the traditional antibiotics needs urgent attention and thus necessitates for the development of the new drug molecules. The use of medicinal plants for the development of the new drug molecule against bacterial infections shows bright future. A wide variety of medicinal plants used traditionally have not yet been systematically investigated against various microbial pathogens.

Plant derived products have shown their beneficial role in the treatment of chronic as well as infectious diseases [105]. Several antibacterial drugs such as ciprofloxacin are available for the treatment of bacterial diseases. However their use is limited for many reasons such as poor solubility, low potency, emergence of resistant strains and the toxicity. Hence, it is necessary to develop new and more effective antibacterial agents.

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*Cardiac Activity and Formulation Evaluation of Isolated Constituents of Piper longum linn*
Formulation studies [106-135]

For any drug molecule to exert better therapeutic activity it is absolutely necessary to formulate it in suitable dosage form so that it can exert desired therapeutic action. Tablets, capsules, syrups, intravenous formulations are some of the examples of dosage forms. Oral drug delivery (Tablets) is the most preferred dosage form due to convenience. In tablet the active ingredient is combined with the excipients as per requirement. Tablets can be prepared by various techniques. Tablets can be prepared by direct compression, wet and dry granulation methods.

In case of directly compressible tablets it can be formed by directly compressing the drug molecules with suitable binders and other excipients (diluent, lubricant etc.) in a tablet compression machine.

In the present study as pipeline is to be isolated which is having low water solubility therefore the cyclodextrin complexation was decided to carry out. The cyclodextrin complexation will change the physicochemical properties of the piperine. Cyclodextrins are ‘bucketlike’ or ‘conelike’ toroid molecules, with a rigid structure and a central cavity, the size of which varies according to the cyclodextrin type. The internal surface of the cavity is hydrophobic and the outside of the torus is hydrophilic; this is due to the arrangement of hydroxyl groups within the molecule. This arrangement permits the cyclodextrin to accommodate a guest molecule within the cavity, forming an inclusion complex. Cyclodextrins may be used to form inclusion complexes with a variety of drug molecules, resulting primarily in improvements to dissolution and bioavailability owing to enhanced solubility and improved chemical and physical stability. From this best complexes will be subjected to formulation in tablet dosage form.

The complexes will be obtained by physical mixture, kneading, solvent evaporation and freeze drying techniques.

The prepared complexes will be subjected for their evaluation for their suitability in formulation studies. The Phase solubility studies can be carried out for understanding the apparent stability constant of complex formed.

Phase solubility study, % drug entrapment, aqueous solubility, dissolution studies, Ultra-violet studies, IR, DSC, XRD etc. will gives idea about the compatibility and suitability of the complexes for the use in formulation of dosage forms.

Dissolution studies of the complex molecule gives an idea for the drug release pattern based on the data the suitable dosage forms can be prepared.

The detailed literature of formulation related work is outlined below. Since cyclodextrin and its derivatives will be used for formulation studies. The reported literature is as follows:

Chao, Yuan et al., (2008) studied the inclusion complex of astaxanthin (ASX) with hydroxypropyl-β-cyclodextrin (HP-β-CD) was prepared. The water solubility of the inclusion complex was >1.0 mg/ml, which is much better than that of ASX. The
stability of the inclusion complex in solution was also tested. Forming of the inclusion complex greatly enhanced the stability of ASX against light and oxygen. Furthermore, the release of ASX from the inclusion complex was controlled.

Tewes, F. et al., (2008) studied complexation efficiency between rifampicin and methylated β-cyclodextrin (RAMEB) or hydroxypropyl-β-cyclodextrin (HPβCD). Rifampicin phase solubility diagrams constructed at pH 9 showed an A_L-type curve for RAMEB and a B_S-type for HPβCD. Stability constants calculated for a 1:1 molar ratio of CD/rifampicin were 73.4 ± 8.2 M\(^{-1}\) for RAMEB and 68.5 ± 5.2 M\(^{-1}\) for HPβCD. Complexes with RAMEB or HPβCD increased 22 times and 7.6 times respectively.

Kuchekar B.S. et al. (2008) studied the solid-state properties, dissolution profile and antimicrobial activity of inclusion complexes of cefdinir (CEF) with β-cyclodextrin (βCD) and hydroxypropyl β-cyclodextrin (HPβCD). The phase solubility profiles of cefdinir with βCD and HPβCD were classified as A_L-type, which indicates the formation of 1:1 stoichiometry inclusion complexes.

Yan, Chunli et al., (2007) studied inclusion complex of (-)-catechin (CA) and (-)-epicatechin (EC) (an enantiomer) to β -cyclodextrins and inclusion complex lead to the improvement in physico-chemical properties.

Figueiras, Ana et al., (2007) reported the formation of the inclusion complex between omeprazole (OME), a benzimidazolic derivative and a methylated cyclodextrin, methyl- β -cyclodextrin (M β CD), with an average degree of substitution of 0.5. The dissolution of OME from the binary systems was studied to select the most appropriate system for the development of a buccal drug delivery formulation. It was concluded that the preparation technique played an important role in the dissolution behaviour of the drug and the inclusion complex between OME and M β CD obtained by spray-drying and freeze-drying allowed better performances.

Jullian, Carolina et al., (2007) studied the slightly water-soluble flavonoid quercetin (QUE) and its inclusion with either β -cyclodextrin (β CD), hydroxypropyl- β -cyclodextrin (HP-β CD) or sulfobutyl ether- β -cyclodextrin (SBE- β CD). All complexes showed a higher scavenging capability with both radicals, compare quercetin in water. Beside, these results indicated that the complexes formed maintained the quercetin antioxidant activity.

Ammara, H.O. et al.,(2006) reported to improve the biological performance of Glimepiride through enhancing its solubility and dissolution rate. Inclusion complexes of glimepiride in β-cyclodextrin (β-CyD), hydroxypropyl- β -cyclodextrin (HP-β-CyD) and sulfobutylether-β-cyclodextrin (SBE-β-CyD), with or without water soluble polymers.

Giuseppina, C. et al.,( 2006) reported the association constant, standard Gibbs energy, enthalpy and entropy for formation of inclusion complexes of propranolol, a β-blocker, with various natural and modified cyclodextrins have been determined by
calorimetry at 298 K. Both natural and methyl-modified α-cyclodextrins do not form complexes, while β and γ-cyclodextrins do. Complexing ability of 2-hydroxypropyl β - cyclodextrin depends on the average substitution degree.

Kale, R. et al., (2005) reported the influence of natural β -cyclodextrin and its hydrophilic derivatives (HP β Cd and SBE7 β Cd) on the in vitro dissolution rate, in vivo absorption and oral bioavailability of a poorly water soluble anti-inflammatory agent, valdecoxib (VALD). Moreover, it was found that in the, the cyclodextrin complexes of drug showed significant improvement in the anti-inflammatory activity.

Ramprakash, G.et al., (2005) has investigated in vivo advantages of a flurbiprofen (FPN)-hydroxypropyl β -cyclodextrin (HP β CD) solid dispersion (SD) in rats, factors affecting the drug release from SD formulations, and pharmacokinetic profile of the drug when administered as SD, in humans. HP β CD enhanced the solubility of the drug, and SD improved bioavailability and reduced ulcerogenicity of the drug in rats. The type of excipient used affected drug release from tablets.

Holvoet,C. et al.,(2005) reported the development of a parenteral lorazepam formulation, using cyclodextrins (CDs) as inclusion complexation agents CDs suitable for parenteral injection, i.e., hydroxypropyl-b-cyclodextrin (HP- β -CD), hydroxypropyl-γ -cyclodextrin (HP- γ -CD), sulfobutylether-7- β -cyclodextrin (SBE-7-β -CD), and maltosyl- β -cyclodextrin (malt- β -CD) for the possibility to increase the solubility of lorazepam. Lorazepam interacted with all tested CD derivatives and 1:1 complexes are formed. HP- β -CD exerts the highest solubility improvement, reaching about 6 mg/ml lorazepam in 30% (w/v) CD solution.

Wen, Xianhong et al., (2004) described an inclusion complex of β -cyclodextrin with carvedilol by using a convenient new method of microwave irradiation. Phase-solubility studies demonstrated the ability of β -cyclodextrins to complex with carvedilol and increase drug solubility. These experimental results confirmed the existence of 1:2 inclusion complex of carvedilol with β -cyclodextrin, the formation constant of complex.

Caira, M. R. et al., (2004) studied the E and Z isomers of the antithrombotic ajoene (4,5,9- trithiadodeca-1,6,11-triene 9-oxide), components of garlic (Allium Sativa, L.), were complexed with heptakis(2,3,6- tri-O-methyl)- β -cyclodextrin (TRIMEB) to yield solid inclusion compounds. Refinement of guest site-occupancies showed that each complex crystal consists of a mixture of diastereomers in 1:1 molar ratio.

Choi, Han-Gon et al., (2003) reported a significant increase in solubility and dissolution rate of nitrendipine, a slightly soluble calcium channel blocker, was achieved by inclusion complexation with hydroxypropyl β-cyclodextrin (HP- β -CD). The solubility of nitrendipine increased linearly as a function of HP- β -CD concentration, resulting in AL-type phase solubility diagram which revealed a formation of inclusion complex in a molar ratio of 1:1, The results indicated that the bioavailability of nitrendipine could be improved markedly by inclusion complexation, possibly due to an increased dissolution rate.
Tonnesen, Hanne Hjorth et al., (2002) reported cyclodextrin complexes of the natural compound curcumin to improve the water solubility and the hydrolytic and photochemical stability of the compound. The hydrolytic stability of curcumin under alkaline conditions was strongly improved by complex formation. The cavity size and the charge and bulkiness of the cyclodextrin side-chains influenced the stability constant for complexation and the degradation rate of the curcumin molecule.

Fernandes, C.M. et al., (2002) studied inclusion complexation between nicardipine hydrochloride (NC), a calcium-channel antagonist, and β-cyclodextrin (β-CD) or hydroxypropyl-β-cyclodextrin (HPβCD). They were evaluated in aqueous environment and in solid state all the combinations with HPβCD were more effective in achieving the enhancement of the NC dissolution rate, yielding better performances than the corresponding ones with βCD.

Tang, B. et al., (2002) studied the supramolecular interaction of curcumin and β-cyclodextrin (β-CD) by spectrophotometry. The results show that β CD reacts with curcumin to form a 2:1 host-guest complex. Based on the enhancement of the absorbance of curcumin produced through complex formation, a spectrophotometric method for the determination of curcumin in bulk aqueous solution in the presence of β-CD was developed.

Wong, J.W. et al., (2001) reported the bioavailability of β- and γ-cyclodextrin artemisinin complexes in comparison with a normal commercially available preparation, Artemisinin 250®. These findings indicated that the β- and γ-cyclodextrin complexes had a much higher rate and extent of bioavailability compared to Artemisinin 250®.

Han-Gon, C. et al., (2001) studied terfenadine-β-cyclodextrin (1 : 2) inclusion complex. For terfenadine, it improved the solubility 200 times and the dissolution rate 5 times. It gave a low histamine level at 30 min, followed by a sustained low level until 60 min, while terfenadine powder gave a low histamine level at 60 min, suggesting that it had faster and more effective antihistaminic activity than terfenadine powder in rats due to fast dissolution and absorption of terfenadine.

Sreenivasan, K. (2001) reported beta cyclodextrin is well known for its ability to form inclusion complexes with a wide class of compounds. The stability of the inclusion complex is largely governed by the hydrophobic nature of the included molecule. The included molecule can be replaced by a relatively more hydrophobic molecule. A simple differential scanning calorimetric (DSC) method is proposed here to study such reaction without the isolation of the complex.

Linares, M.S. et al., (2000) reported the complexation of 2-hydroxy-N-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine with a highly soluble cyclodextrin, hydroxypropyl-b-cyclodextrin (HP-β-CD) in aqueous media by solubility methods. Inclusion took place with 1:1 stoichiometry. The stability constant of the complexes calculated from the slope and the intercept of the phase solubility diagrams are larger in the less ionized form, whereas greater overall solubility is obtained in basic media.
Pina, M. E. et al., (2000) reported the role of β -cyclodextrin (β -CD) on the apparent solubility of theophylline by the solubility method. Binary systems of theophylline and β-CD were prepared using the dry co-grinding method. It was concluded that β -CD is related to an increase in the apparent solubility and dissolution rate of the drug, promoting improvement on the release of theophylline from matrices manufactured with hydroxypropylmethylcellulose (HPMC).

Plessing, Rossel, et al., (2000) suggested complexation between acyclovir (ACV), an antiviral drug used for the treatment of herpes simplex virus infection, and β -cyclodextrin (β -CD) was studied in solution and in solid states. Solubility of ACV in solid complexes was studied by the dissolution method and it was found to be much more soluble than the uncomplexed drug.

Blanchard, J. et al.,(2000) reported increase in the solubility of phenytoin by complexing it with varying concentrations of 2-hydroxypropyl- β -cyclodextrin (HPBCD) and create an entirely aqueous formulation with a pH significantly closer to physiologic pH (7.4). The phenytoin-HPBCD complexation was characterized using phase-solubility analysis at HPBCD concentrations ranging from 10 to 50% w/v over the pH range of 7.4–11.0

Miyake, K., et al., (2000) reported enhancement in the solubility, dissolution rate, and oral bioavailability of rutin by complexation with 2-hydroxypropyl- β -cyclodextrin (HP- β -CyD). The dissolution rates of rutin increased by the complexation with CyDs in the order of rutin alone < HP- β CyD ≤ β -CyD.

Mura, P. et al., (1999) reported the influence of the preparation method (physical mixing, ball-milling, kneading, sealed-heating) on the physicochemical properties of the products. Kneading and sealed heating techniques led to amorphous products in the case of systems with methyl- β -cyclodextrin, whereas crystalline drug was still clearly detectable in all products with β-cyclodextrin. Independently of the preparation technique, all combinations with methyl- β -cyclodextrin yielded better performance than the corresponding ones with β -cyclodextrin.

Ahmed, M.O. et al., (1998) reported improvement of solubility and release rate of the clotrimazole is therefore rapid antimycotic activity. Hence, the effect of cyclodextrins (CDs) on the physicochemical properties and antimycotic activity of clotrimazole was studied. The coevaporate of clotrimazole with DM- β -CD showed higher dissolution rate in good correlation with the solubility data, and these reflect the higher antimycotic activity by rapid diffusion through agar medium. Both physical mixture and inclusion complex of clotrimazole with DM- β -CD were formulated as effervescent vaginal tablets; they found to possess an excellent antimycotic activity.

Moyano, J.R. et al., (1997) evaluated solid complexes between gliclazide and cyclodextrin were prepared by methods like kneading, coprecipitation, neutralization, co-grinding and spray-drying. The complexes, obtained by neutralization and spray-drying methods, showed enhanced dissolution rates of gliclazide.
Veiga, F. et al., (1996) studied inclusion complexes of tolbutamide with β-cyclodextrin and hydroxypropyl-β-cyclodextrin by using methods like: kneading, coprecipitation and freeze-drying. The dissolution rate of tolbutamide from the inclusion complexes was much more rapid than tolbutamide alone.

Esclusa-Diaz, M.T. et al., (1996) reported increase in poor buffer pH 5 and 6 solubility of ketoconazole was studied. Two systems were used: binary complexes prepared with β-cyclodextrin and multicomponent systems (β-cyclodextrin and an acid compound), obtained by spray-drying. The solubility of ketoconazole increased significantly with the cyclodextrin complexes.
2.5 REFERENCES


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