SUMMARY AND CONCLUSION
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The main purpose of the present work was to establish a relation for therapeutic activity of the plant *Piper longum linn* in the cardiac diseases. Literature survey was carried out to review different plants which are used in the Ayurveda for the treatment of the cardiac related problems. It has been found that the plant *Piper longum linn* has been used as spice and the medicinal uses of the plant were also documented not only in the ancient literature but also in modern science. Piperine which is one of the major constituent of the plant *Piper longum linn* has been indicated for its use as bioavailability enhancer.

The plant was authentified and standardized as per the official methods reported in the literature. Various standardized parameters like foreign matter, total ash, acid-insoluble ash, water soluble ash, extractive values and loss on drying. Then plant *Piper longum linn* was selected for studying its pharmacological activity in different animals.

For pharmacological activities it was necessary to find median lethal dose of the plant extract. Hence root extract of the plant was prepared in different solvents like petroleum ether, water and alcohol. The dose of the plant extract as well as the major isolated constituent (Piperine) was suitably prepared and then the LD_{50} was calculated. The extracts and piperine were found to be safe up to 1000 mg/kg of the body weight of the rats used.

The activity of the extracts of the plant were studied on the isolated frog heart. The pet ether extract and piperine exhibited significant activity and blocked the actions of adrenaline at 200 μg/ml. The activity of this plant has been found to be on the beta receptor of the isolated frog heart. In the presence of propranolol also synergistic effect was observed and hence it can be said that the plant might be acting through the activation of beta receptor.

Since the pet ether extract and isolated piperine has given maximum activity in isolated frog heart experiment, pet ether extract was selected for further activity studies.

Column chromatographic techniques was used to isolate the bioactive fraction of the plant *Piper longum linn*. Thin layer chromatographic techniques was used to select the best suitable solvent for the isolation. Extracts were subjected to hot and room temperature analysis and the solvent in which maximum number of the spots obtained was selected for the isolation of the constituents. Pet ether extract was chromatographed on column and then subjected for the isolation. Different fractions were collected and pooled together and depending upon the R_{f} values they were subjected for identification. It has been found to contain Piperine as major constituent. Piperine was hence selected for different pharmacological studies along
with the pet ether extract. The structure of the isolated constituent was confirmed by spectral techniques like IR, NMR, $^1$H NMR and GC-MS.

Cardiac enzyme study was carried out to study the effect of pet ether extract and piperine at biochemical level. The piperine as well as pet ether extract was administered in rats orally and then the values of myocardial marker enzymes (CK, LDH, AST and ALT) in normal as well as isoproterenol induced necrotic rats was compared. It has been observed that the plant and piperine pre treatment maintained the normal levels of the cardiac markers in ischemic conditions. The p values for the serum and heart tissue in the necrotic heart was found to be significant and hence it can be said that the pet ether extract as well as piperine has the protective effects in the myocardial ischemia.

The lipid peroxides and glutathione levels were also measured. Pet ether extract and piperine pretreatment decreases lipid peroxide level and maintain glutathione content to near normal.

The pet ether extract and piperine also found to inhibit free radical scavenging activity in the DPPH method. The significant values of the activity are obtained and are comparable with the standard Vitamin C.

Histopathological studies were performed and it has been found that the pet ether extract as well as piperine both are useful in the content uniformity at cellular level. Maximum tissue damage was observed in isoproterenol treated animals. Pet ether extract and piperine treatment however maintains the integrity at the cellular level.

In antibacterial studies it was found that pet ether extract inhibit the growth of microorganism at effective concentration of 10 mg/well. The isolated and purified constituent piperine was found to inhibit growth of microorganism at effective concentration of 2 mg/well.

The isolated constituent piperine was subjected to formulation development. To change the physicochemical properties especially solubility of piperine it was first complexed with $\beta$-cyclodextrin and HP-$\beta$-cyclodextrin. The apparent 1:1 stability constants were in the order HP-$\beta$CD > $\beta$CD. The complexation was done by using four methods physical mixture, coevaporation/solvent evaporation, kneaded method, freeze drying/lyophilization. The inclusion-complexes were characterized by XRD, DSC and IR. Lyophilized complexes gave clear cut idea about the strong inclusion complex formation as well as increase in solubility and dissolution profile. These inclusion complexes were subjected for the preparation of tablet dosage form. The dosage form evaluated for all their parameters were meeting the pharmacopoeial standards. It has been observed that the release pattern of the piperine is significantly improved in the inclusion complexes form as well as in tablet dosage form.