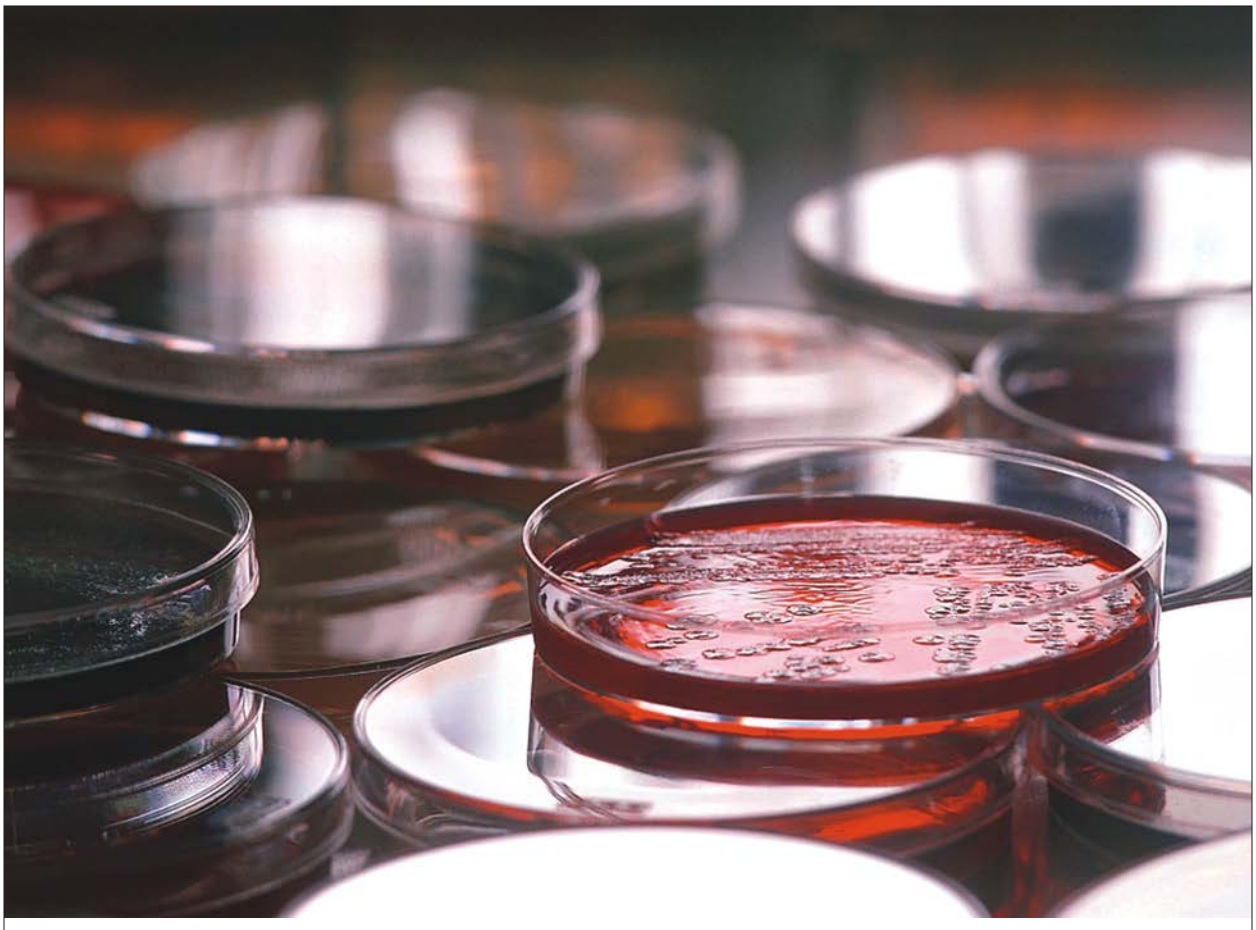

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Chronotropic and Inotropic Effects of *Piper longum* Linn

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Abstract: Extract of the plant *Piper longum* Linn (Family: Piperaceae) is used as folk medicine in India and China. Roots of the plant *Piper longum* are reported for the treatment of heart diseases in ancient literature of East Asia. However, no data is available on exact role of fractions of plant in heart diseases. In the present study we have isolated four major fractions namely F, G, H and I and the activity of the fractions was studied on isolated frog heart. Fraction F has produced negative inotropic and negative chronotropic effect on isolated frog heart at 200 μg mL^{-1}. Fraction F shows maximum antagonistic activity on beta-receptors by decreasing heart rate and force of contraction. Actions of Adrenaline are blocked by fraction F and in the presence of propanolol, it shows continuous decrease in heart rate and force of contraction. In the present study, it was observed that fraction F shows antagonistic activity on beta adreno-receptors.

Key words: Chronotropic, inotropic, beta- blocking, *Piper longum*

Introduction

Medicinal plants have shown tremendous potential for the development of new drug molecules for various serious diseases. Many plant derived products have found to play an important role in various cardiovascular diseases (Charksamhita and Stanza, 1984). *Piper longum* Linn. (Piperaceae) has been used as a therapeutic agent in the treatment of various pathological conditions (Kirtikar and Basu, 1984). Fruits of the plant have been used as thermogenic, stomachic, aphrodisiac, carminative, expectorant, laxative, digestive, emollient, anti-girdiasis, anti-amoebic, anti-asthamatic, antiseptic and in combination with Butea Monosperma the plant showed immuno-stimulatory activity in mice (Warrier *et al*., 1995; Agarwal *et al*., 1997; Goshal *et al*., 1996; Reddy *et al*., 1984). Dry fruits of the *Piper longum* have been used in the prevention of recurrence of asthma (Dahanukar and Karandikar, 1984). The benzene extract of *Piper longum* when mixed with methanol extract of the Embelia ribes berries and administered to female rats inhibited pregnancy in 80% of the animals indicating Anti-fertility activity of the fruits (Kholkute *et al*., 1979).

Alkaloidal amides of the plant shows enhancement in the bioavailability of therapeutically important drugs (Khajuria *et al*., 1998). *Piper longum* in combination with the extract of *Zingiber officinalis* and *Ferula* species are found to be effective in gastric ulceration (Agarwal *et al*., 2000). As a fraction of Brahmi Rasayana *Piper longum* shows Anti-inflammatory activity (Jain *et al*., 1994). Root of the plant have been used traditionally by Indians for regularizing the heart rate and it is used as tonic for heart diseases (Charksamhita and Stanza, 1984).

Phytochemical investigation of the plant showed presence of piperlongumine, piperlonguminine, piperine, sesamin, 3,4,5-trimethoxycinnamate, beta-sitosterol, aristolactum, piperyline, piplartine and hexacosanoic acid isobutyl amide (Dutta *et al*., 1977; Shankaracharya *et al*., 1997; Desai *et al*., 1989; Koul *et al*., 1988).

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The diverse pharmacological properties of the *Piper longum* has indicated its vital role as a therapeutic agent in many diseases. Roots of the plant have shown good therapeutic potential. However proper qualification and quantitative analysis of the plant is very essential for exploring the exact role of fractions in serious cardiac diseases. Literature survey shows that no data is available on the cardiovascular properties of fractions of *Piper longum*. Since heart diseases has posed a great challenge in front of us it has become essential to discover plant based drugs which are safe and very useful in cardiac diseases. Fruit and roots of different species of piper like *Piper aduncum* (Orjala *et al*., 1993), *Piper betle* (Nair and Bruke, 1990) and *Piper sarmentosum* have been used as a food material in Asian countries which indicate its safety.

The root of the plant shows beneficial role in the treatment of cardiac disease however no data is available on the exact role of the different fractions of *Piper longum*. Hence, the aim of present study was to isolate different fractions of *Piper longum* by column chromatographic technique (Wu *et al*., 2004) and to screen the exact cardiac activity of different isolated fractions. Dried roots of *Piper longum* were powdered and hexane was used as eluent for the isolation of different fractions. The isolated fractions were subjected for studying their cardiac effect on isolated frog heart (Satoskar and Bhandarkar, 1985).

Materials and Methods

Isolation

Dried roots of *Piper longum* Linn. were purchased from local supplier and authentified from botanical survey of India, Pune (Voucher No. 2005/13). The dried roots were powdered and then thin layer chromatographic analysis was carried out in different solvents such as water, benzene, acetone, ether, ethyl acetate, methanol, hexane and chloroform. Depending upon R_f values and number of spots on TLC plate the solvent which extracts the maximum number of the fractions was selected. Hexane has shown presence of maximum ten spots on TLC plate and hence was selected for isolation (Table 1). TLC plates were observed under Ultra-violet light chamber and iodine chamber. The dried powdered roots were extracted with hexane at room temperature for 48 h. This extract was then filtered and evaporated under reduced pressure to obtain a viscous mass (4 g). This material was chromatographed over silica gel using hexane as eluent (Lee *et al*., 2002 ; Yang *et al*., 2002).

Total four major Fractions, namely F, G, H and I along with some minor fractions were isolated. The compounds obtained were purified with crystallization and no further column chromatography was needed since on gas chromatography fractions showed single fraction.

Cardiac Activity

The study involves screening of large number of compounds of *Piper longum*, which are not screened earlier for cardiac activities. Roots of *Piper longum* have been used as an active extract in various ayurvedic formulations and preparations. Due to the beneficial role of pharmacological actions of *Piper longum* in cardiac diseases, we have selected roots of the plant for the isolation and cardiac studies of different fractions. On TLC plate total ten fractions were observed. In order to study the cardiac activity of such large numbers of fractions we have selected isolated frog heart experiment.

Table 1: Number of fractions observed on thin layer chromatography

Solvent	No. of constituents
Water	02
Benzene	03
Acetone	03
Ether	07
Ethyl acetate	03
Methanol	03
Hexane	10
Chloroform	05

Frogs (*Rana tigrina*), weighing 150-200 g were pithed (Guede-Guina *et al*., 1995; Bhansali *et al*., 1987; Tripathi and Das, 1983). The heart was exposed and isolated from body after cannulation of the inferior vena cava using symes canula. The canula was connected to the reservoir containing frog's ringer solution. The solution was continuously bubbled with air, at room temperature. The composition of frog ringer in mM was Na^+ 110.7, Cl^- 114.2, K^+ 1.2, Ca^{++} 1.10, HCO_3^- 2.8, $H_2PO_4^-$ 0.1 and glucose 11.1 (pH 7.6-8.0).

The heart was stabilized for fifteen minutes prior to the administration of drugs. Responses were recorded on a smoked drum using a starling heart lever. The study was conducted in six different groups of animals. The fractions were prepared in different doses like 05, 10, 20, 30, 40, 50, 100 and 200 μg mL^{-1}. The activity of fractions was tested in the presence of Adrenaline and Propranolol. The crude hexane extract which is a mixture of all the fraction was also prepared in similar doses as that of the individual fraction and was subjected to cardiac studies on isolated frog heart. Similar condition is maintained in all the experiments to compare the activity of different fractions. This experiment is very reproducible, economic and can be used as a cardiac activity marker.

Results and Discussion

The results were analyzed statistically using students 't' test. Heart rate and force of contraction are mentioned in terms of percentage (Table 2). Initially the normal responses of isolated frog heart were recorded on smoked drum. Adrenaline was administered in doses of 05, 10, 20, 40 and 100, 200 μg mL^{-1}., the increase in heart rate and force of contraction indicate beta adrenergic agonist activity of adrenaline (Satoskar and Bhandarkar, 1985). When Fraction F was administered in presence of adrenaline, in doses like 5, 10, 20, 30, 40, 100 and 200 μg mL^{-1}. There was a dose dependent decrease in heart rate and force of contraction. As the dose of the fraction was increased the heart rate and force of contraction decreases which indicates that the maximum inhibition is observed at a higher dose. At 200 μg mL^{-1} maximum inhibition of adrenaline activity was observed (Fig. 1). Since adrenaline is a agonist of beta receptor in order to confirm the beta antagonistic activity, of the fraction F, atropine was administered in similar doses as that of fraction F, to verify the action of fraction F on cholinergic receptor. It was observed that in presence of Atropine the actions of Fraction F remains unaffected which indicates that the actions of Fraction F are mediated through beta receptor. The crude extract which is a combination of the all fractions was compararitively, less effective on the heart rate and force of contraction. The extract shows maximum inhibition of heart rate and force of contraction at a increasing dose of 500 μg mL^{-1}. The finding suggests that the fraction F acts as antagonist of beta-receptor. Propranolol, which is a beta-blocking agent was used as a standard drug for comparison of beta antagonistic activity. Propanolol was also administered in a doses like 5, 10, 20, 30, 40, 50, 100 and 200 μg mL^{-1}. It has been observed that the fraction F in presence of propranolol shows synergic action and decreases the heart rate and force of contraction. The fraction F thus shows antagonistic activity on beta receptors and could be used as beta blocking agent.

Table 2: Cardiac activity of fraction F and crude extract on isolated frog heart

Dose	Fraction F (Presence Adrenaline)		Fraction F+Propranolol		Extract	
(μg mL^{-1})	HR (%)	FC (%)	HR (%)	FC (%)	HR (%)	FC (%)
05	94	61	80	76	98	66
10	96	50	78	60	98	66
20	80	45	69	52	90	60
40	72	40	65	38	88	56
100	70	30	54	20	60	40
200	65	20	40	18	66	36
500	ND	ND	ND	ND	45	38

Each value was obtained from six experimental groups. Each group consisted of five animals. $p<0.01$. (HR% = Heart Rate in Percentage average, FC% = Force of contraction in Percentage average; ND = Not Done)

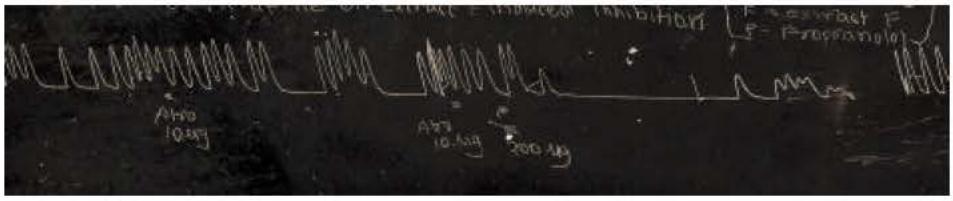

Fig. 1: effect of fraction F on isolated frog heart

Beta blockers has been established as a standard first line therapy for both stable and unstable angina pectoris. Several studies and clinical trials have shown that beta blockers is used to reduce mortality when used in patients after myocardial infarction and in heart failure. In a recent literature, it has been pointed out that the currently available beta-blockers have deleterious effects on two aspects of clinical relevance to ischemic heart diseases, namely coronary collateral flow under controlled occlusion of the coronary artery and the impact on ischemic and stunned myocardium (Sato *et al*., 2004; Billinger *et al*., 2004; Monnet *et al*., 2004).

Thus there is a urgent need to find out new, safe and efficient drug molecules as beta blocking agents to fight against heart diseases. The existing synthetic Beta blockers are having certain adverse reaction like indigestion, nausea, diarrhoea. Since, *Piper* species are used as food material they might not show adverse reactions as that of present synthetic beta blocking agents. The data indicate that the isolated fractions can be used for their beta antagonistic activity in cardiac diseases.

Similar studies were carried out on fractions G, H and minor fractions however they have not produced any significant effects on isolated frog heart. The study of Fraction I is under progress. The spectral analysis of the fraction F is $IR_{\nu\ max}$ KBr cm^{-1}:2941, 1683, 1620, 1132. ^1H NMR ($CDCl_3$) δ:3.90 (9H), δ: 4.1 (2H br), δ:1.6 (6H), δ:1.4 (10H), δ:5.9 (2H), δ:6.8 (3H). MS m/z (Rel. Int.):238 (M^+), 237, 146, 119, 92. The spectral data shows that one of the fraction F is having structural similarity as that of Piperine.

Conclusions

Fraction F produces negative inotropic and negative chronotropic effect on isolated frog heart. Fraction F blocks the actions of adrenaline completely at 200 mcg mL^{-1}. The heart rate and force of contraction decreases in a dose dependent manner. Fraction F acts as antagonist of beta adreno-receptors. It blocks the actions of Adrenaline in a dose dependant manner. The hexane extract of root of *Piper longum* was comparatively found to be less active than fraction F. The isolated fraction was thus found to have more activity and the present work can serve as a platform for studying the beta antagonistic activity of the isolated fractions of the *Piper longum*. The biochemical role of the fraction can be investigated in future in animals like, mice and rat which may explore the role of the fraction F as a beta blocking agent.

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References

Agarwal, A.K., D.M. Tripathi, R. Sahai, N. Gupta, R.P. Saxena, A. Puri, M. Singh, R.N. Misra, C.B. Dubey and K.C. Saxena, 1997. Management of giardiasis by a herbal drug 'Pipalli Rasayana: A clinical study. J. Ethnopharmacol., 56: 233-236.

Agarwal, A.K., C.V. Rao, K. Sairam, V.K. Joshi and R.K. Goel, 2000. Effect of *Piper longum* Linn., *Zingiber officinalis* Linn. and *Ferula* species on gastric ulceration and secretion in rats. Ind. J. Exp. Biol., 38: 994-998.

Bhansali, B.B., S. Vyas and R.K. Goyal, 1987. Cardiac effects of quercetin on the isolated rabbit and frog heart preparations. Ind. J. Pharmacol., 19:100-107.

Billinger, M., L. Raeber and C. Seiler, 2004, Coronary collateral perfusion in patients with coronary artery disease: Effect of metoprolol. Eur. Heart J., 25: 565-570.

Charksamhita and S. Stanza, 1984. Jadaraji Trikamji Acharya (Ed.), Varanasi, Chawkhambha Sanskrit Sanathana, pp: 29.

Dahanukar, S.A. and S.M. Karandikar, 1984. Evaluation of antiallergic activity of *Piper longum*. Ind. Drugs, 21: 377-383.

Desai, S.J., R.N. Chaturvedi, L.P. Bodheka and N.B. Mulchandani, 1989. Aristrolactams and 4,5-dioxoaporphines from Indian *Piper* species. Ind. J. Chem., 28: 775-777.

Dutta, C.P., N.Banerjee, A.K. Sil and D.N. Roy, 1977. Studies on the genus *Piper* : Studies on the roots of *Piper longum* Linn. Ind. J. Chem., 15B: 583-584.

Goshal, S., B.N. Prasad and V. Lakshmi, 1996. Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica in vitro* and *in vivo*. J. Ethnopharmacol., 50: 167-170.

Guede-Guina, F., C.S. Tsai, M.O. Smith, M. Vangah-Manda, B. Washington and R.F. Ochillo, 1995. The use of isolated functional heart to pharmacologically characterize active ingredients in the aqueous extracts of *Mareya micrantha*. J. Ethnopharmacol., 45:19-26.

Jain, P., N.K. Khanna, N. Trehan, V.K. Pendse and J.L. Godhwani, 1994. Anti-inflammatory effects of an Ayurvedic preparation, *Brahmi rasayan*, in rodents. Ind. J. Exp. Biol., 32: 633-636.

Khajuria, A., U. Zutshi and K.L. Bedi, 1998. Permeability characteristics of Piperine on oral absorption-An active alkaloid from peppers and a bioavailability enhancer. Ind. J. Exp. Biol., 36: 46-50.

Kholkute, S.D., M.B. Kekare and S.R. Munshi, 1979. Anti-fertility activity of *Piper longum* in female rats. Ind. J. Exp. Biol., 17: 291.

Kirtikar, K.R. and B.D. Basu, 1984. *Piper longum* Linn., Indian Medicinal Plants. Periodical Expert Book Agency, New Delhi, 3: 21-28.

Koul, S.K., S.C. Taneja, V.K. Agarwal and K.L. Dhar, 1988. Minor amides of *Piper* species. Phytochemistry, 27: 3523-3527.

Lee, S.E., N.E. Mahoney and B.C. Campbell, 2002. Inhibition of aflatoxin B1 biosynthesis by piperlongumine isolated from *Piper longum* L. J. Microbiol. Biotechnol., 12: 679-682.

Monnet, X., P. Colin and B. Ghaleb, 2004. Heart rate reduction during exercise-induced myocardial ischaemia and stunning. Eur. Heart J., 25: 579-586.

Nair, M.G. and B.A. Bruke, 1990. Antimicrobial *Piper* metabolite and related compounds. J. Agric. Food Chem., 38: 1093-1100.

Orjala, J., C.A.J. Erdelmeir, A.D. Wright, T. Rai and O. Sticker, 1993. Five new prenylated p-hydroxy benzoic acid derivatives with antimicrobial and molluscicidal activity from *Piper aduncum*. Planta Med., 59: 546-551.

Reddy, M.K., C.K. Kokate and K.M. Rao, 1984. Antifertility activity of crude drug combinations in albino rats 1. Ind. Drugs, 21: 533-535.

Sato, M., S.E. Harding and P.A. Poole-Wilson, 2004. B-blockers, myocardial ischaemia and collateral circulation. Eur. Heart J., 25: 537-539.

Satoskar, R.S. and S.D. Bhandarkar, 1985. Adrenergic and Adrenergic Blocking Drugs. In: Pharmacology and Pharmacotherapeutics. Satoskar, R.S. and S.D. Bhandarkar (Eds.). 9th Edn., Bombay: Popular Prakashan, pp: 215.

Shankaracharya, N.B., L.J. Roa, J.P. Naik and S. Nagalakshmi, 1997. Characterization of Indian long pepper (*Piper longum* Linn.) J. Food Sci. Technol., 34: 73.

Tripathi, H.C. and P.K. Das, 1983. Interactions of digoxin with autonomic drugs in isolated frog heart. Ind. J. Pharmacol., 15: 279-291.

Warrier, P.K., V.P. K Nambiar and K.C. Raman, 1995. *Piper longum* Linn. Indian Medicinal Plants, Orient Longman Ltd., Madras, 4: 290.

Wu, S., C. Sun, S. Pei, Y. Lu and Y. Pan, 2004. Preparative isolation and purification of amides from the fruits of *Piper longum* L. by upright counter-current chromatography and reversed-phase liquid chromatography. J. Chromatograp., A 1040: 193-204.

Yang, Y.C., S.G. Lee, H.K. Lee, M.K. Kim, S.H. Lee and H.S. Lee, 2002. A piperidine amide extracted from *Piper Longum* L. fruit shows activity against Aedes aegypti mosquito larvae. J. Agric. Food Chem., 50: 3765-3767.