DOI: 10.5567/pharmacologia.2013.335.342

Pharmacological Characterization of Pp-17, a α/β-adrenoceptor Blocking Agent with Antihypertensive Effect

[1]Sumit Chaudhary, [1]Aakanksha Dube, [1]Prasad Thakurdesai, [1]Subhash Bodhankar and [2]Poonam Piplani

[1]Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune -411 038, India

[2]University Institute of Pharmaceutical Sciences, Punjab University, Chandigarh 160 014

ABSTRACT

Background: The purpose of the current study was to investigate the various pharmacological characteristics of a newly synthesized PP-17 [3-(2-hydroxy-3-isopropylamino-propoxy)-benzaldehyde oxime] under the *in vitro* and *in vivo* conditions. **Methods:** Potency of PP-17 was investigated towards different α, β-adrenoceptor subtypes by using rat isolated right atria, uterus, distal colon, thoracic aorta preparations. Antihypertensive and metabolic activity was tested in left renal artery ligated and fructose induced hypertension model. **Results:** pA_2 values of PP-17 for β_1, β_2, β_3 and α_1 adrenoceptor were 7.0±0.1, 5.7±0.1, 6.0±0.1 and 7.0±0.1, respectively. The β_1/β_2 selectivity ratio was calculated and shows in the order of atenolol >PP-17 >labetalol >propranolol while β_1/α_1 selectivity ratio was in the order of labetalol >PP-17. PP-17 (10 and 30 mg kg^{-1}, p.o.) significantly decreased the mean arterial blood pressure and heart rate in both left renal artery ligated and fructose induced hypertension model. In addition, treatment with PP-17 (30 mg kg^{-1}, p.o.) showed significant decrease in plasma TG and VLDL level. **Conclusion:** Our results suggest that PP-17 is a dual α_1 and β_1-adrenoceptor receptor antagonist having antihypertensive property.

Key words: Adrenoceptor receptor, fructose, mean arterial blood pressure, heart rate

Pharmacologia 4 (4): 335-342, 2013

INTRODUCTION

Hypertension is a circulatory disease characterized by sustained elevation of blood pressure. It is often defined as mild (borderline) or severe depending on the blood pressure levels. This disease can be genetic in origin (also termed primary or essential) or may occur as a secondary product of cardiac diseases such as congenial heart diseases or interactions with environmental factors, such as a high salt diet[1].

β-Adrenoceptor antagonists are widely used in the treatment of hypertension and coronary heart disease. For the treatment of cardiovascular diseases, they may generally be divided into first, second and third-generation agents. The classic first-generation agents such as propranolol or timolol are nonselective for β_1 or β_2-receptors and have no obvious ancillary cardiovascular effects. Second-generation compounds are those that exhibit selectivity for β-receptor subtypes, such as β_1-selective compounds atenolol, metoprolol and betaxolol[2]. The term "third-generation β_1-blocker" refers to the kind of blocking drugs that possess an ancillary cardiovascular action other than β-blockade. Examples include the β-blocker/vasodilator agent's labetalol and carvedilol[3]. Both labetalol and carvedilol are vasodilators because of α-adrenergic blockade. Labetalol is characterized by similar affinities for α_1 and β_1-receptors. Carvedilol is also a α_1-blocking agent but with approximately 2 to 3 fold selectivity for β_1-versus α_1-receptors. Nevertheless, this degree of α_1-blockade is responsible for the moderate vasodilating properties of carvedilol[4].

During the past few years, studies demonstrated that most antihypertensive agents modify insulin sensitivity in parallel with alterations in the atherogenic lipid profile. α_1-blockers and angiotensin converting enzyme inhibitors were found to improve insulin resistance and the profile of atherogenic lipids, whereas most of the calcium channel blockers were found to be metabolically inert. The diuretics and β-adrenoreceptor antagonists further decrease insulin sensitivity and worsen

Corresponding Author: Sumit Chaudhary, Department of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune-411 038, India Tel: 91-20-25437237 Fax: 91-20-25439383

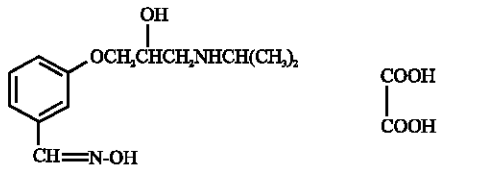

Fig. 1: Chemical structure of PP-17

dyslipidemia[5]. The mechanisms by which β-adrenoreceptor antagonist treatment exert its side effects are not fully understood but several possibilities exists such as significant body weight gain, reduction in enzyme activities (muscle lipoprotein lipase and lecithin cholesterol acyltransferase), alterations in insulin clearance and insulin secretion and, probably most important, reduced peripheral blood flow due to increase in total peripheral vascular resistance[6]. Recent metabolic studies found beneficial effects of the newer vasodilating β-blockers, such as dilevalol, carvedilol and celiprolol, on insulin sensitivity and the atherogenic risk factors. In many hypertensive patients, elevated sympathetic nerve activity and insulin resistance are deleterious combination. Although conventional β-blocker treatment was able to take care of the former, the latter got worse; the newer vasodilating β-blocker generation seems to be capable of successfully treating both of them[7].

These advantages have initiated the search for some novel potential vasodilating β-blocker of greater selectivity toward β_1-receptor which also improves insulin sensitivity and dyslipidemia. We have been involved in development of new β-blockers for past few years[8, 9]. Recently we developed new chemical entity namely PP-17, having a propanolamine group at para position in benzaldehide oxime ring. PP-17 [3-(2-hydroxy-3-isopropylamino-propoxy)-benzaldehyde oxime] (Fig. 1), was synthesized from combination of m-hydroxybenzaldehyde and aryloxypropanol (the basic structure with β-blocking activity). This study was aimed to investigate the various pharmacological characteristics of PP-17 in different animal model of hypertension.

MATERIALS AND METHODS

Animals: Adult Wistar rats (100-250 g) of either sex were purchased from National Toxicology Center, Pune, India. Animals were housed in clean environment under 12:12 light:dark cycle at a temperature of 25±2°C and relative humidity of 55±5%. Food and water were available *ad libitum*. All procedures involving animals were approved by the Institutional Animal Ethical Committee of Poona College of Pharmacy.

β_1-adrenoceptor assay: Male Wistar rats were euthanized by cervical dislocation under isoflorane anesthesia. Pericardium over the heart was carefully removed from the heart and the right atria were dissected. A suture was tied to the upper end and lower tip of the atria. The atria was mounted in a 25 mL organ bath filled with Krebs bicarbonate buffer solution containing (mM) NaCl (118), KCl (4.7), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25), Disodium EDTA (0.03) and glucose (11.1). Temperature of physiological solution was maintained at 37°C and aerated with carbogen (95% O_2 and 5% CO_2). Spontaneous responses of atria were recorded by connecting the upper end to the force transducer (T-305) connected to student physiograph (Bio-Devices, Ambala, India). The resting tension was maintained at 0.5 g during a 30 min equilibration period. Positive chronotropic effect of isoprenaline was determined in the presence and absence of PP-17 or atenolol or propanolol or labetalol at different concentration. Sinus rate was assessed for 15 sec after the addition of each successive concentration of isoprenaline for 1 min[9].

β_2-adrenoceptor assay: Female Wistar rats having estrus phase were used to isolate uterine horns. Uterine horn was mounted in organ baths (INCO, Ambala, India) filled with 25 mL of Locke ringer solution of composition (in mM) NaCl (154), KCl (5.6), $NaHCO_3$ (6.0), $CaCl_2$ (2.2), glucose (11.1), ascorbic acid 30 μM and sodium salt of EDTA 30 μM. Temperature of physiological solution was maintained at 37°C and aerated with carbogen (95% O_2 and 5% CO_2). To achieve a steady spontaneous contraction, an initial tension of 2 g was applied for 30 min. After the tissue was equilibrated, relaxant effect of isoprenaline was determined in the presence and absence of PP-17 or atenolol or propanolol or labetalol at different concentration[10].

β_3-adrenoceptor assay: Male Wistar rats were used to isolate distal colon (2.5 cm in length). Distal colon was mounted in 25 mL organ baths (INCO, Ambala, India) containing Krebs bicarbonate buffer solution of compostion (mM) NaCl (118), KCl (4.7), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25), Disodium EDTA (0.03) and glucose (11.1). Temperature of physiological solution was maintained at 37°C and aerated with carbogen (95% O_2 and 5% CO_2). Krebs bicarbonate buffer solution was also contained 30 μM of ascorbic acid, 30 μM of sodium salt of EDTA to prevent oxidation of catecholamine and 1 μM of prazosin hydrochloride to remove any possible contribution from α adrenoceptor. Tissue was allowed to equilibrate for 30 min before starting the experimental procedures. Initially 50 mM KCl was added and allowed to stabilize for 15 min. After 30 min of resting period tissue was contracted with a submaximal concentration of KCl (30 mM) and allowed

to stabilize. Relaxant effect of isoprenaline was determined in the presence and absence of PP-17 or atenolol or propanolol or labetalol at different concentration[11].

α_1-Adrenoceptor assay: Thoracic aorta from male wistar rats were dissected and cleaned to remove fat and connective tissue. Endothelium was removed by gently rubbing the intimal surface with fine wires. The 3 mm length ring was mounted under 0.5 g of resting tension in organ baths containing Krebs solution of the following composition (mM): NaCl (118), KCl (4.7), $CaCl_2$ (1.25), KH_2PO_4 (1.14), $MgSO_4$ (1.1), glucose (10), $NaHCO_3$ (25). Temperature of physiological solution was maintained at 37°C and aerated with carbogen (95% O_2 and 5% CO_2). Tissue was allowed to equilibrate for 60 min with periodic washes before starting experiments. Removal of functional endothelium was checked by the lack of any relaxation to acetylcholine (1 mM) in rings pre-contracted with noradrenaline (0.1 mM). Vasocontraction effect of phenylephrine was determined in the presence and absence of PP-17 or atenolol or propanolol or labetalol at different concentration[12].

***In vivo* evaluation of β-adrenoreceptor antagonistic activity:** Wistar rats were anaesthetized with urethane (1.25 g kg^{-1}, i.p.). Jugular vein was canulated for administration of PP-17 or atenolol or labetalol and Carotid artery was cannulated for blood pressure measurement. Body temperature was maintained at 37°C with a help of thermal blanket. The carotid artery was connected to pressure transducer (SS13L, BIOPAC Systems, Inc., Santa Barbara, CA) for measurement of MABP and heart rate. Dose response curve of isoprenaline was constructed for increase in heart rate (tachycardia) and fall in mean arterial blood pressure (MABP) after intravenous injection (0.3, 1 and 3 μg kg^{-1}) in presence and absence of PP-17 or atenolol or labetalol[9].

Left renal artery ligated hypertension: Male Wistar rats (175-200 g) were anesthetized with Ketamine (75 mg kg^{-1}, i.p.) and Xylaxine (10 mg kg^{-1}, i.p.) and Left Renal Artery (LRA) was ligated. After renal ligation, the animals were housed and provided with 1% sodium chloride solution instead of normal drinking water. Animals were divided into following 7 groups after 4 weeks of LRA ligation. Group I: normal control animals, Group II: vehicle (0.05% Tween-80+0.5% CMC, 3 mL kg^{-1}). Group III: PP-17, 3 mg kg^{-1}, Group IV: PP-17, 10 mg kg^{-1}, Group V: PP-17, 30 mg kg^{-1}, Group VI: atenolol, 10 mg kg^{-1} and Group VII: labetalol, 10 mg kg^{-1}. All treatments were given orally for next 2 weeks. At the end of 6th week, MABP and Heart rate was recorded by cannulating the carotid artery as previously described[13].

Fructose induced hypertension: Wistar rats (200-220 g) were divided into seven groups according to body weight. Normal control group was given ordinary drinking water *ad libitum* throughout the whole treatment course and the remaining group was given 10% fructose solutions to drink *ad libitum*[14]. Nine weeks later, the fructose-treated animals were assigned the following treatment regimen; Group I: normal control animals, Group II: vehicle (0.05% Tween-80+0.5% CMC, 3 mL kg^{-1}). Group III: PP-17, 3 mg kg^{-1}, Group IV: PP-17, 10 mg kg^{-1}, Group V: PP-17, 30 mg kg^{-1}, Group VI: atenolol, 10 mg kg^{-1} and Group VII: labetalol, 10 mg kg^{-1}. Vehicle, PP-17 and drug treatment was initiated after 9 weeks of fructose feeding and treatment was continued for next 2 weeks. At the end of 11th week, MABP and Heart rate was recorded by cannulating the carotid artery as previously described.

Measurement of mean arterial pressure and heart rate: Wistar rats were anaesthetized with urethane (1.25 g kg^{-1}, i.p.). Carotid artery was cannulated with the help of polyethylene catheter containing heparin dissolved in isotonic saline (50 IU mL^{-1}). Body temperature was maintained at 37°C with a help of thermal blanket. The cannulated artery was connected to pressure transducer (SS13L, BIOPAC Systems, Inc., Santa Barbara, CA) for measurement of MABP and heart rate.

Biochemical measurements: Concentrations of glucose, cholesterol, LDL, HDL, VLDL and triglycerides were measured in plasma samples at the end of the experiment using a Hitachi 902 fully automated random access biochemistry analyzer Roche. Plasma insulin was determined with an ELISA (linco research).

Statistical analysis: Data in table and figure are expressed as Mean±Standard Error of Mean (SEM). One-way Analysis of Variance (ANOVA; Graph Pad PRISM®, Version 4.0, San Diego, CA, USA) followed by Dunnett's test was applied to determine differences between the groups. A value of $p<0.05$ was considered significant.

RESULTS

β-Adrenoceptor blockade and selectivity: PP-17 antagonized the isoproterenol induced positive chronotropic effects on the isolated wistar rat right atria. PP-17 also caused a dose-dependent parallel shift to the right of the isoproterenol concentration response curves yielding pA_2 values of 7.0±0.2 (Fig. 2a). PA_2 values of

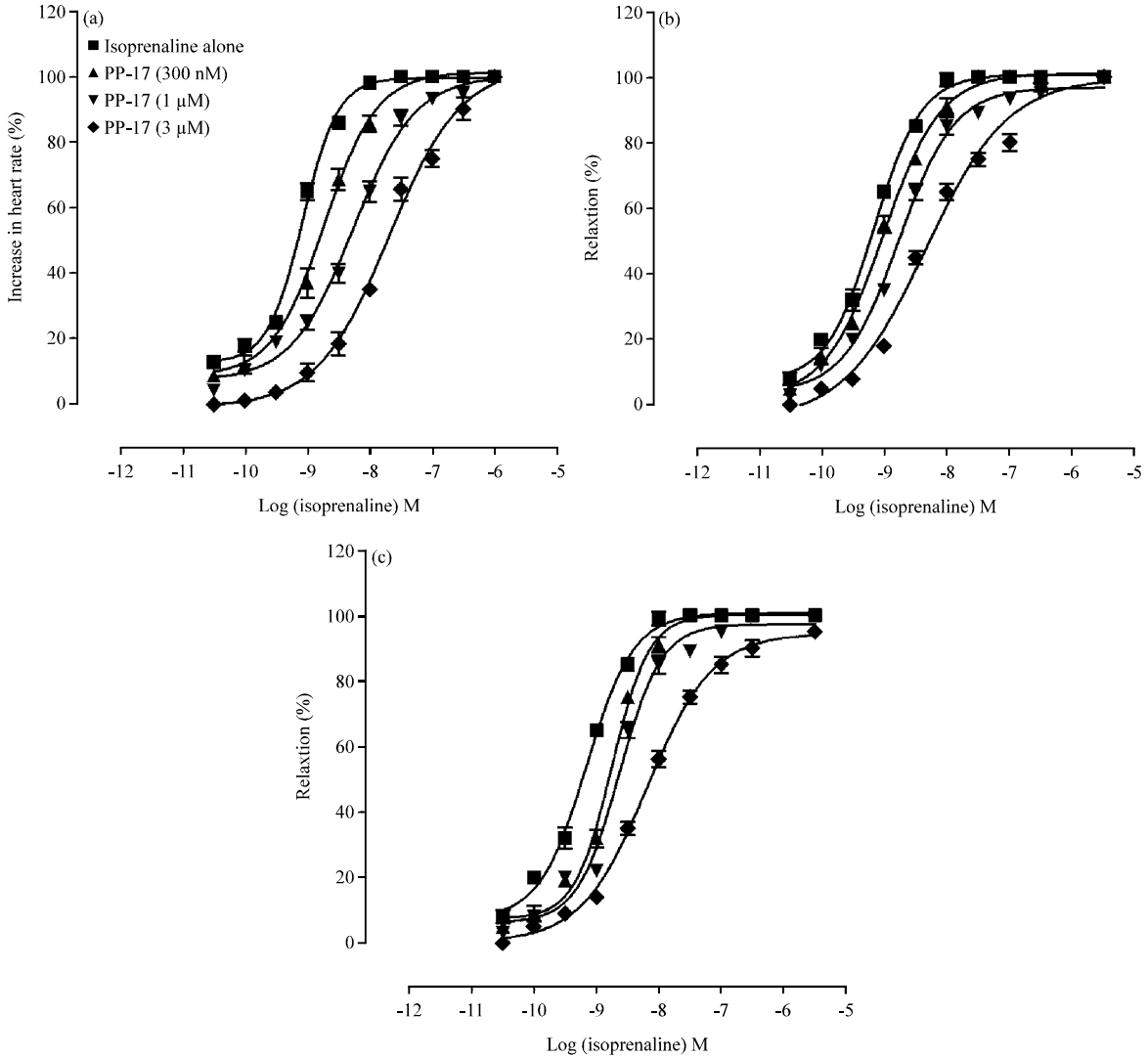

Fig. 2 (a-c): Effects of PP-17 on responses in Wistar rat atria, uterus and colon. Mean cumulative concentration–response curves are shown for the (a) positive chronotropic responses to isoprenaline in spontaneously beating rat right atrium, (b) relaxant effects of isoprenaline in rat uterus (c) relaxant effects of isoprenaline in rat colon in the absence or presence of PP-17. Each point represents the mean ±S.E.M. of six individual experiments.

PP-17, atenolol, labetalol and propranolol treatment, calculated from Schild plots are shown in Table 1. In all cases, the slopes of Schild plots were not significantly different from 1.0. PP-17 antagonized isoprenaline induced relaxation of spontaneously contracting uterus and KCl induced contraction in rat colon. PP-17 also caused a rightward shift of concentration response curve of isoprenaline in isolated rat uterus and isolated rat colon indicating β_2 and β_3 adrenoreceptor antagonistic activity (Fig. 2b,c). The apparent pA_2 values of PP-17 for β_2 and β_3 adrenoreceptor were found to be 5.7 ±0.1 and 6.0 ±0.1 respectively (Table 1). The β_1/β_2-selectivity ratio was calculated from the antilogarithm of the difference between the pA_2 values obtained from rat right atria and uterus. The estimated β_1/β_2-selectivity ratio value (20.0) indicated that PP-17 had more affinity to β_1 adrenoreceptor than to β_2 adrenoreceptor subtypes. The relative order of β_1/β_2-selectivity was found to be in the order of Atenolol > PP-17 > Labetalol > Propranolol.

α_1-Adrenoceptor blocking activity: PP-17 antagonized phenylephrine induced vasoconstrictions effects in

Table 1: α and β-Adrenoreceptor blocking potency and selectivity of PP-17

	α & β blocker	pA_2 values	Selectivity ratio				
	β_1 Right atria (Slope)	β_2 Uterus (Slope)	β_3 Colon (Slope)	α_1 Aorta (Slope)	β_1/β_2	β_1/α_1	β_1/β_3
PP-17	7.0±0.2 (1.0±0.4)	5.7±0.1 (1.01±0.1)	6.00±0.1 (0.9±0.1)	7.0±0.1 (0.9±0.1)	20	1	10
Prapanolol	8.3±0.1 (1.0±0.2)	8.2±0.1 (1.0±0.3)	6.6±0.2 (0.9±0.2)	NT (1.0±0.2)	1	-	50
Atenolol	7.2±0.1 (0.9±0.1)	5.6±0.1 (1.0±0.1)	4.6±0.3 (1.0±0.1)	NT (1.0±0.1)	40	-	398
Labetalol	7.9±0.1 (1.0±0.1)	7.1±0.1 (1.1±0.2)	6.5±0.2 (1.0±0.1)	7.5±0.2 (1.0±0.1)	6	3	25

The selectivity ratio values were obtained from the antilogarithm of the difference between the mean pA_2 values from in vitro studies. The pA_2 values were obtained from the formula pA_2= [log (DR-1) ×log molar concentration antagonist] and the slope values were calculated from individual Schild plot by regression analysis. Data are expressed as Mean±SEM, n = 6 at each individual experiments. NT: Not Tested

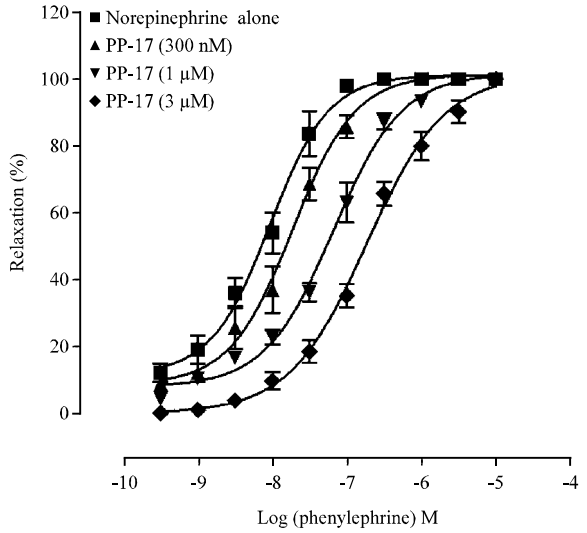

Fig. 3: Inhibitory effects of PP-17 on the concentration–response curves of phenylephrine for the contractions in rat aorta strips. Data are expressed as Mean±SEM, n = 6 at each individual experiments

isolated Wistar rat thoracic aorta rings. PP-17 also caused a dose-dependent parallel shift to the right of the phenylephrine concentration–response curves (Fig. 3). The apparent pA_2 value for PP-17 on aorta rings was 7.0±0.1 as indicated in Table 1.

***In vivo* evaluation of β-adrenoceptor antagonistic activity:** Administration of isoprenaline (0.3, 1 and 3 μg kg^{-1}, i.v.) produced a dose dependent increase in heart rate and decrease in the MABP. Administration of isoprenaline (0.3, 1 and 3 μg kg^{-1}, i.v.) in the presence of PP-17 (10 and 30 μg kg^{-1}, i.v.), labetalol (30 μg kg^{-1}, i.v.) and atenolol (30 μg kg^{-1}, i.v.) significantly reduced isoprenaline induced increase in heart rate (Fig. 4a) and decrease in the MABP (Fig. 4b). Inhibition of

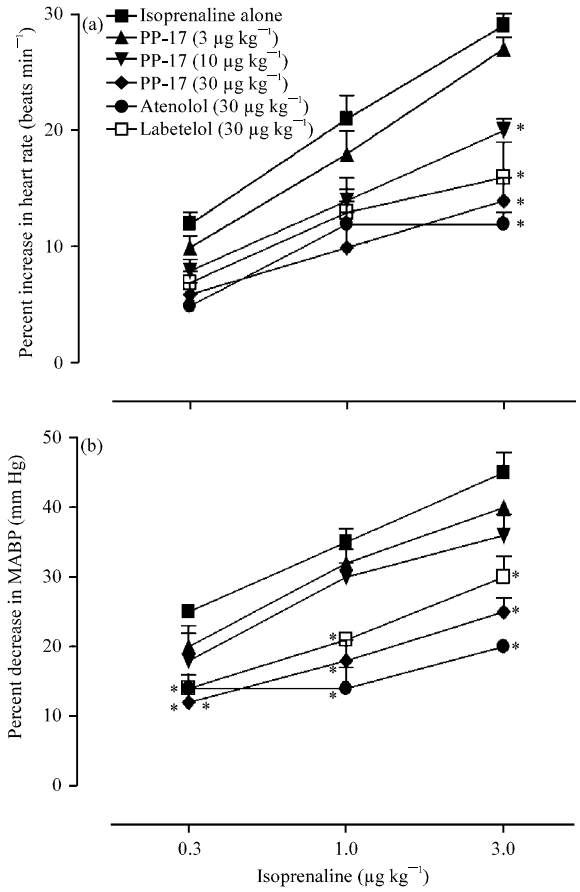

Fig. 4(a-b): Percent increase in heart rate (a) and % decrease in MABP (b) in normal rats with isoprenaline alone or with PP-17, atenolol and labetalol. Data are expressed as mean±SEM, n = 6 at each individual experiments. *p<0.05 as compared to Isoprenaline group

cardioaccelerator and hypotensive responses to isoprenaline by PP-17 revealed β adrenoreceptor blocking activity.

Table 2: Effect of PP-17 on mean arterial blood pressure, heart rate, plasma levels of glucose, insulin, cholesterol, triglycerides, HDL, LDL and VLDL in Fructose -fed rat

	Control	Vehicle	PP-17 (3 mg kg^{-1})	PP-17 (10 mg kg^{-1})	PP-17 (30 mg kg^{-1})	Atenolol (10 mg kg^{-1})	Labetalol (10 mg kg^{-1})
MABP (mm Hg)	95±4.3	155±3.7#	149±3.0	141±2.4*	105±2.1*	105±4.5*	110±1.5*
Heart rate (Beats min^{-1})	309±12	338±13	326±11	306±10	282±5.2	279±7.5*	295±2.5
Glucose (mg dL^{-1})	98±10	140±8#	130±8	125±7	122±11	140±8	129±2.5
Insulin (ng mL^{-1})	1.2±0.2	3.8±0.3#	3.6±0.2	2.9±0.4	2.8±0.2	3.7±0.6	3.1±0.8
Cholesterol (mmol L^{-1})	1.3±0.10	1.6±0.04	1.5±0.07	1.4±0.04	1.2±0.08	1.6±0.08	1.3±0.09
Triglycerides (mmol L^{-1})	0.8±0.01	1.5±0.03#	1.4±0.02	1.3±0.02	1.1±0.03*	1.6±0.07	1.4±0.04
HDL (mmol L^{-1})	0.38±0.01	0.35±0.04	0.36±0.02	0.37±0.02	0.42±0.03	0.31±0.02	0.41±0.03
LDL (mmol L^{-1})	0.38±0.02	0.58±0.04#	0.53±0.02	0.49±0.02	0.49±0.03	0.56±0.06	0.46±0.05
VLDL (mmol L^{-1})	0.36±0.03	0.66±0.04#	0.59±0.04	0.55±0.04	0.48±0.02*	0.50±0.02	0.50±0.01

Data are expressed as Mean±SEM, n = 8 at each individual treatment, #p<0.05 as compared to control group, *p<0.05 as compared to vehicle group

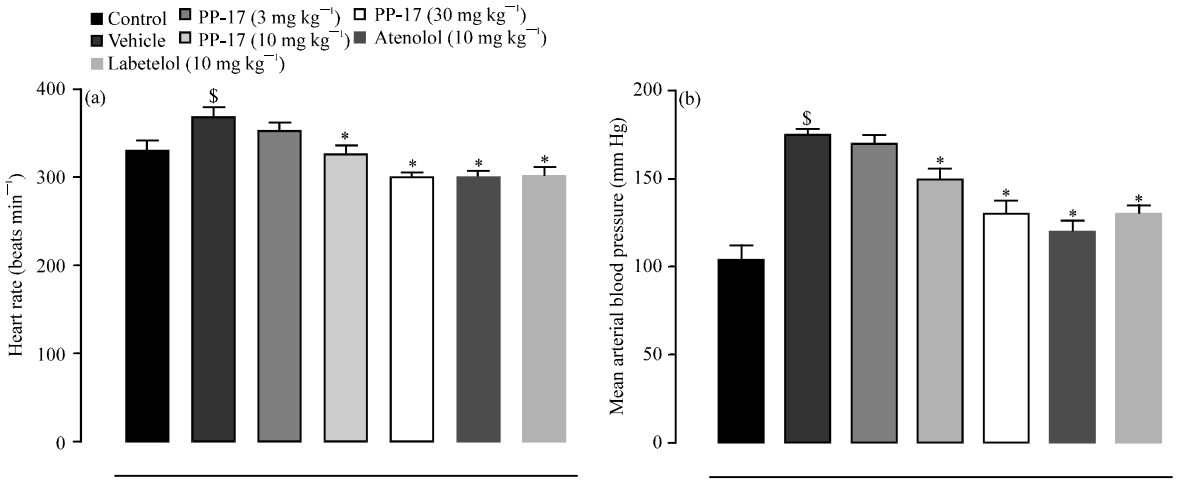

Fig. 5(a-b): Effect of PP-17 (3,10 and 30 mg kg^{-1}, p.o.), atenolol (10 mg kg^{-1}, p.o.) and labetalol (10 mg kg^{-1}, p.o.) on Mean Arterial Blood Pressure (MABP) and heart rate of LRA ligated rats after 14 days of treatment. (a) Change in mean arterial blood pressure. (b) Change in heart rate. Data are expressed as mean±SEM, n = 8 at each individual treatment. $P<0.05 as compared to normal control group, *p<0.05 as compared to vehicle group

LRA ligated hypertension: The MABP and heart rate significantly increased in LRA ligated vehicle group when compared with normal control group. Treatment with PP-17 (3, 10 and 30 mg kg^{-1}, p.o.) produced a dose dependent decrease in MABP and heart rate of LRA ligated rats. PP-17 (10 and 30 mg kg^{-1}, p.o.), labetalol (10 mg kg^{-1}, p.o.) and atenolol (10 mg kg^{-1}, p.o.) showed significant decrease in MABP and heart rate. The fall in MABP produced by PP-17 (10 mg kg^{-1}, p.o.) was less than the atenolol (10 mg kg^{-1}, p.o.) and labetalol (10 mg kg^{-1}, p.o.) shown in Fig. 5.

Fructose induced hypertension: Plasma concentrations of glucose, insulin, cholesterol, LDL, HDL, VLDL and TG levels was estimated after 12 h fasting in normal and fructose fed rats (Table 2). After 11 weeks, fructose-fed rats showed significant increase in plasma glucose, insulin, TG, LDL and VLDL level when compared with normal control rats. Treatments with PP-17 (10 and 30 mg kg^{-1}, p.o.) showed significant decrease in MABP as compared with vehicle group. Treatment with PP-17 (30 mg kg^{-1}, p.o.) showed significant decrease in plasma TG and VLDL level as compared with vehicle group.

DISCUSSION

This study demonstrates that PP-17 is dual a dual α_1 and β_1-adrenoceptor receptor antagonist. PP-17 significantly decreased blood pressure and heart rate in high renin and fructose induced hypertensive model. The similarities between the PP-17, atenolol and labetalol end with their cardiovascular effects, however; their metabolic effects are different. PP-17 showed more decrease in fasting plasma glucose and insulin levels than labetalol whereas atenolol did not showed any change in metabolic parameters. This indicates that PP-17

improves insulin sensitivity more than labetalol whereas atenolol did not. The results of this study indicated that PP-17 differ from classical β blocker.

Isoprenaline induced increase in the atrial rate was competitively antagonized by PP-17, labetalol, atenolol and propranolol, suggesting that PP-17 possessed β_1-adrenoreceptor antagonistic activity. The rank order potency for β_1 adrenoceptor was found to be atenolol >PP-17 >Labetalol >Propranolol. The presence of vast majority of β_2-adrenoceptor in rat uterus was confirmed by autoradiography localization and the length of gestation influenced the β2-adrenoceptor mediated response[10]. PP-17 weakly antagonized relaxation response of isoprenaline in spontaneous contraction of uterus indicates that PP-17 possessed weak β_2-adrenoceptor antagonistic activity. The presence of β_3-adrenoreceptor has been confirmed in human and rodent colon[11]. PP-17 weakly antagonized relaxation response of isoprenaline on KCl mediated contraction in rat colon suggesting that PP-17 possessed weak β_3 antagonistic activity. The relative order of β_1/β_2-selectivity was found to be in the order of Atenolol>PP-17>Labetalol>Propranolol.

The effect of PP-17 against α_1-adrenoceptor was evaluated with the thoracic aorta isolated from the wistar rats. In isolated rat aorta, PP-17 was found to competitively antagonize increases in contraction induced by phenylephrine, suggesting that PP-17 possessed α_1-adrenoceptor antagonist activity.

Isoprenaline is a potent β-adrenoreceptor agonist with high affinity for all β adrenoceptor, devoid of any action on α-adrenoceptor. Intravenous infusion of isoprenaline stimulates cardiac β-adrenoceptor and lowers peripheral vascular resistance[9]. The tachycardia produced by isoprenaline is primarily due to the β_1 adrenoceptor activity in heart and the hypotensive effect is primarily due to the β_2 adrenoceptor in the blood vessels. In our study, administration of isoprenaline produced tachycardia and hypotension in anesthetized rats.

PP-17 (10 μg kg^{-1}, i.v.) blocked isoprenaline induced tachycardia without affecting blood pressure which indicate that PP-17 having selective β-adrenoceptor (β_1) antagonistic activity this dose levels. However, PP-17 (30 μg kg^{-1}, i.v.) blocked both tachycardial and hypotensive effect of isoprenaline, suggesting blockade of both β_1 and β_2-adrenoceptor. In vivo experiments suggested that PP-17 possess cardio selective β_1-blockade at lower dose levels and selectivity was abolished at higher doses.

In rat, constriction of renal artery with an intact contralateral kidney produces hypertension with an elevated plasma renin activity (PRA). β-blockers decreases blood pressure by different mechanisms such as reduction in the cardiac output, reduction of renin release from the juxtaglomerular cells, central action reducing sympathetic activity, change in the baroreceptor sensitivity, an alteration in peripheral adrenergic neuron function and an increase in the prostacyclin biosynthesis[13]. In the present investigation, PP-17 also showed significant antihypertensive effect in this model that was less than the atenolol and labetalol.

The results of this study indicate that insulin resistance, hyperinsulinemia, increase in mean blood pressure and hypertriglyceridemia develop when normal rats are fed a high fructose in drinking water, as has been seen previously[15,16]. In addition, we have shown that these metabolic changes are associated with an increase in LDL and VLDL level in blood plasma. Several antihypertensive drugs effectively prevent and reverse the increase in blood pressure induced by high fructose diets[17].

Hyperinsulinemia and insulin resistance could induce elevation of blood pressure levels via a variety of mechanisms including sodium-water retention, sympathetic nerve stimulation, changes in transmembrane ion traffic and direct stimulation of smooth muscle cell growth[15]. Moreover, other reports have shown that reducing insulin levels in these rats leads to a reduction in blood pressure and correction of other metabolic abnormalities[18,19]. Fructose diets have been found to activate sympathetic nervous system activity and elevate blood pressure in rats[20,21]. Sympathoexcitation also may play an early and integral role in the final expression of elevated plasma insulin levels and blood pressure in rats fed with a high fructose diet[22]. Plasma norepinephrine has been found elevated in sucrose- and fructose-fed rats and it has been proposed to account for hypertension and insulin resistance because of its vasoconstrictor activity[23]. PP-17 (10 and 30 mg kg^{-1}, p.o.) significantly decreased the MABP and heart rate in fructose-fed animals. In addition, PP-17 (30 mg kg^{-1}, p.o.), significantly decreased TG and VLDL level as compared with vehicle treated rats.

The above result indicates PP-17 reduced the extent of development of hypertension induced by the left renal artery ligation and high fructose by blocking both α_1 and β_1-adrenoceptor receptor. PP-17 also improved lipid profile in fructose fed rats. Therefore, PP-17 might have beneficial effects on blood pressure without unwanted side effect.

In conclusion, this study indicates that PP-17 is a third-generation selective β_1-adrenoceptor blocker with vasorelaxant characteristics, which may be explained by its α_1-adrenoceptor blockade. PP-17 was different from such first-generation compounds as atenolol and propranolol in that it possessed ancillary vasorelaxing

activity. However, further refinement of our understanding of these cardiovascular mechanisms will be required to extend these findings to the treatment of hypertension.

REFERENCES

1. Bohm, M., P. Gierschik, A. Knorr, U. Schmidt, K. Weismann and E. Erdmann, 1993. Cardiac adenylyl cyclase β-adrenergic receptors and G proteins in salt-sensitive hypertension. Hypertension, 22: 715-727.
2. Duval, N., C.R. Lee, M.T. Eon, P. Petruzzo and S.Z. Langer, 1998. The beta-1-adrenoceptor antagonist betaxolol is not released from the heart of the anaesthetized dog during sympathetic nerve stimulation. Br. J. Pharmacol., 95: 683-688.
3. Bristow, M.R., 1993. Pathophysiologic and pharmacologic rationales for clinical management of chronic heart failure with beta-blocking agents. Am. J. Cardiol., 71: C12-C22.
4. Bristow, M.R., R.L. Roden, B.D. Lowes, E.M. Gilbert and E.J. Eichhorn, 1998. The role of third-generation beta-blocking agents in chronic heart failure. Clin. Cardiol., 12: I3-I13.
5. Jacob, S., K. Rett and E.J. Henriksen, 1998. Antihypertensive therapy and insulin sensitivity: Do we have to redefine the role of β-blocking agents? Am. J. Hypertension, 11: 1258-1265.
6. Szabo, T., S. von Haehling and W. Doehner, 2008. Weight change with beta-blocker use: A side effect put into perspective. Am. J. Med., 121: e15-e15.
7. Bhatt, L.K., K. Nandakumar, S.L. Bodhankar, J. Bansal and P. Piplani, 2007. Beta-blocking activity of PP-34, a newly synthesized aryloxypropanolamine derivative and its cardioprotective effect against ischemia/reperfusion injury in laboratory animals. J. Pharm. Pharmacol., 59: 429-436.
8. Nikam, A.P., S.L. Bodhankar, P. Piplani, J. Bansal and P.A. Thakurdesai, 2008. Beta-adrenoreceptor blocking and antihypertensive activity of PP-24, a newly synthesized aryloxypropanolamine derivative. J. Pharm. Pharmacol., 60: 1501-1506.
9. Nandakumar, K., S.K. Bansal, R. Singh, A.J. Mohite and S.L. Bodhankar *et al.*, 2005. Study of β-adrenoceptor antagonistic activity of DPJ 904 in rats. Pharmacology, 74: 1-5.
10. Levy, B. and S. Tozzi, 1963. The adrenergic receptive mechanism of rat uterus. J. Pharmacol. Exp. Therap., 142: 178-184.
11. Brahmadevara, N., A.M. Shaw and A. MacDonald, 2003. Evidence against β_3-adrenoceptors or low affinity state of β_1-adrenoceptors mediating relaxation in rat isolated aorta. Br. J. Pharmaco., 138: 99-106.
12. Huang, Y.C., B.N. Wu, Y.T. Lin, S.J. Chen, C.C. Chiu, C.J. Cheng and I.J. Chen, 1999. Eugenodilol: A third-generation β-adrenoceptor blocker, derived from eugenol with α-adrenoceptor blocking and β_2-adrenoceptor agonist-associated vasorelaxant activities. J. Cardiovascular. Pharmacol., 34: 10-20.
13. Cangiano, J.L., C. Rodriguez-Sargent and M. Martinez-Maldonado, 1979. Effects of antihypertensive treatment on systolic blood pressure and renin in experimental hypertension in rats. J. Pharmacol. Expt. Ther., 208: 310-313.
14. Dai, S. and J.H. McNeill, 1995. Fructose-induce hypertension in rat is concentration and duration-dependent. J. Pharmacol. Toxicol. Methods, 33: 101-107.
15. Dimo, T., J. Azay, P.V. Tan, J. Pellecuer, G. Cros, M. Bopelet and J.J.Serrano, 2001. Effects of the aqueous and methylene chloride extracts of Bidens pilosa leaf on fructose-hypertensive rats. J. Ethnopharmacol., 76: 215-221.
16. Dimo, T., S.V. Rakotonirina, P.V. Tan, J. Azay, E. Dongo and G. Cros, 2002. Leaf methanol extract of Bidens pilosa prevents and attenuates the hypertension induced by high-fructose diet in Wistar rats. J. Ethnopharmacol., 83: 183-191.
17. Rosen, P., P. Ohly and H.Gleichmann, 1997. Experimental benefit of moxonidine on glucose metabolism and insulin secretion in fructose-fed rat. J. Hypertens., 15: S31-S38.
18. Verma, S., S. Bhanot and J.H. McNeill, 1994. Antihypertensive effects of metformin in fructose-fed hyperinsulinemic, hypertensive rats. J. Pharmacol. Exp. Ther., 271: 1334-1337.
19. Bhanot, S. and J.H. McNeill, 1996. Insulin and hypertension: A causal relationship? Cardiovasc. Res., 31: 212-221.
20. Fournier, R.D., C.C. Chiueh, I.J. Kopin, J.J. Knapka, D. DiPette and H.G. Preuss, 1986. Refined carbohydrate increases blood pressure and catecholamine excretion in SHR and WKY. Am. J. Physiol., 250: E381-E385.
21. Young, J.B. and L. Landsberg, 1977. Stimulation of the sympathetic nervous system during sucrose feeding. Nature, 269: 615-617.
22. Baron, D., 1993. 5 Cardiovascular actions of insulin in humans. Implications for insulin sensitivity and vascular tone. Baillieres Clin. Endocrinol. Metab., 7: 961-987.
23. Verma, S., S. Bhanot and J.H. McNeill, 1999. Sympathectomy prevents fructose-induced hyperinsulinemia and hypertension. Eur. J. Pharmacol., 373: R1-R4.