

STUDY OF BETA-ADRENORECEPTOR BLOCKING AND ANTIHYPERTENSIVE ACTIVITY OF A NEWLY SYNTHESIZED ARYLOXYPROPANOLAMINE DERIVATIVE, PP-28 IN LABORATORY ANIMALS.

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Summary

Beta-adrenoceptor blockers are an important class of drugs used in management of patient with cardiovascular diseases. These drugs have been shown to reduced mortality in hypertension. However, the main side effect of these drugs is due to antagonism of β_2 -adrenoceptors in the airways, resulting in bronchospasm. Therefore, more cardio selective beta-blockers have been developed to offer a lower side effect profile. We have studied a new aryloxypropanolamine derivative (PP-28) with more cardio selectivity and efficacy against hypertension in rats. Oxalate salts of 1-tert-butylamino-3-[3-(tert-butylamino-methyl)-phenoxy]-propan-2-ol. (PP-28) is a novel beta-adrenoceptor antagonist. In-vitro studies in rat isolated right atria, rat uterus and rat distal colon preparations were carried out to investigate the potency of PP-28 towards different beta-adrenoceptor subtypes. The pA₂ values of PP-28 for β_1 , β_2 , and β_3 adrenoceptor were 7.69+/-0.10, 6.03+/-0.08 and 6.10+/-0.14, respectively. The β_1/β_2 selectivity ratio calculated was in the order of PP-28 > atenolol > propranolol. PP-28 also blocked significantly isoprenaline induced tachycardia and hypotensive responses in anesthetized rat. Antihypertensive effect of PP-28 was observed in left renal artery ligated (LRA) and 10% Fructose - induce hypertensive rat. Treatment with PP-28 (1, 3 and 10 mg/kg, orally) produced a dose dependent decrease in MAP and heart rate of LRA ligated rats as well as 10% Fructose - induce hypertensive rat. The efficacy of PP-28 was found to be greater then atenolol.

In conclusion, PP-28 is a cardioselective beta-adrenoceptor antagonist, possessing potent Antihypertensive effects in left renal artery ligated and 10% Fructose - induce hypertensive rat

Keywords: Left renal artery ligated (LRA), β -Adrenergic blocking activity, Hypertension.

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Introduction

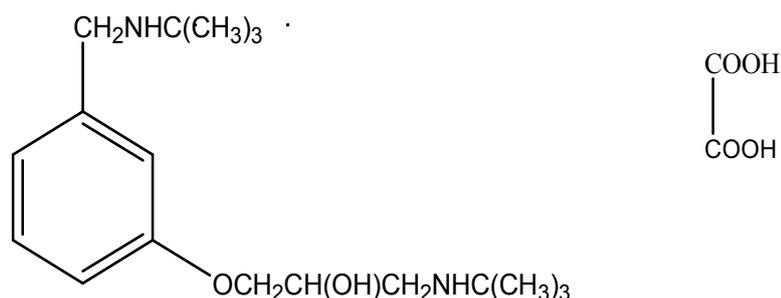
Hypertension is a circulatory disease characterized by sustained elevation of blood pressure. It is often defined as mild (borderline) or severe depending on the blood pressure levels. The disease can be genetic in origin (also termed primary or essential) or may occur as a secondary product of either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

The fact that primary hypertension has a genetic component prompted association studies relating its manifestation with the prevalence of certain polymorphisms in genes coding for proteins such as β -AR receptors and angiotensin-converting enzyme (ACE) known to be involved in cardiac function. Studies addressing the functional expression of β -AR have produced inconsistent results, with some suggesting variations in adrenoreceptor responses as being dependent on population or ethnic variables (2). Some studies found no changes in lymphocyte β -AR density in essential hypertension (3). However, although hypertension per se may have no direct influence on β -AR signaling, receptor desensitization has been implicated in the pathophysiology of hypertension as well as progression from hypertrophy to heart failure (4). β -AR alterations occur secondary to blood pressure elevation regardless of whether hypertension is genetic and that the mechanisms regulating adrenoreceptor responsiveness on prolonged agonist exposure may be altered in hypertension, thereby contributing to the pathophysiology of the disease.

β -Blockers retain their position among the basic therapies for numerous cardiovascular and non-cardiovascular condition. Such β -Blockers share a common feature of competitive antagonism on β -adrenoreceptors. They differ in many aspects of pharmacological properties such as potency, selectivity of β_1 to β_2 receptor, intrinsic sympathomimetic activity (5). In this type of study we use rat right atria and rat uterus to estimate selectivity of test agent between β_1 to β_2 receptor in rational animal model. Additionally isolated rat colon tissues were used to evaluate the test agents for predominant β_3 adrenergic receptor sites. β -adrenergic antagonist and particularly those without cardiac selectivity carry considerable risk in patient with bronchospastic disease because they may block β_2 receptor in therapeutic doses. In airway system, application of β -adrenergic receptor blocker with tracheal relaxant activity may reduce the risk of asthmatic attack in patients with bronchospastic disease. However pulmonary effect such as dyspnea, airway obstruction and pulmonary failure has also occurred in the administration of β -adrenergic receptor blocker. (6, 7). These facts have led many investigators to search for newer β -adrenergic receptor blocking agents that would lessen the adverse effects. Therefore β blockers with more selectivity are emerging.

These advantages have initiated the search for some novel potential cardio-selective β -blockers of greater selectivity toward β_1 receptor with other additional advantage (such as vasodilatation). We have been involved in development of new β -blockers for past few years, recently we develop new β -blocker of same series PP-28 chemically known as 1-tert-butylamino-3-[3-(tert-butylamino-methyl)-phenoxy]-propan-2-oloxlate [8, 9, 10].

In this research, PP-28 chemically known as 1-tert-butylamino-3-[3-(tert-butylamino-methyl)-phenoxy]-propan-2-oloxlate. (Figure 1), was synthesized from Tert-butyl amine, which was combined with aryloxypropanol (the basic structure with β -blocking activity). Basically, this study was aimed to investigate the various pharmacological characteristics of PP-28, under in vivo and in vitro conditions, including its ability β adrenoceptor antagonism and its antihypertensive activity.



1-tert-butylamino-3-[3-(tert-butylamino-methyl)-phenoxy]-propan-2-ol-oxalate.

Figure1: Chemical structure of PP-28.

Materials and methods

Drug

Isoprenaline hydrochloride (Sigma Chemical Company, St. Louis, MO, USA), urethane ((Fluka Chemika GmbH, Buchs, France), propranolol hydrochloride, atenolol hydrochloride (Nicholus Piramal India limited, Goregaon (East), Mumbai, India) were dissolved or diluted in physiological saline to appropriate concentrations. All other chemicals required for preparation of physiological salt solution were of analytical grade purchased from S.D. Fine Chemicals Ltd., Mumbai, India.

Animals

Adult Wistar rats (200 –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 $55 \pm 5\%$. Food and water were available ad libitum. The Institutional Animal Ethics Committee of Poona College of Pharmacy approved all the experimental protocols for the study.

In vitro studies

β_1 -adrenoceptor assay

Male Wistar rats were euthanased by cervical dislocation. Pericardium over the heart was carefully removed from the heart and the right atrium was dissected. A suture was tied to the upper end and lower tip of the atrium. The atria was mounted in a 25 ml organ bath filled with Krebs' bicarbonate buffer containing (mM) NaCl 118, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25, di sodium EDTA 0.03 and glucose 11.1. The solution was kept at 37°C and was aerated with carbogen. 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 minutes equilibration period. Cumulative CRCs for increase in sinus rate (positive chronotropic effect) of isoprenaline were constructed by addition of log increment dose of isoprenaline at an interval of 3 minutes.

Sinus rate was assessed 15 seconds after the addition of each successive concentration of isoprenaline for 1 minute. For assessment of antagonist activity, response of atrium to isoprenaline dose was determined in presence of PP-28 or Atenolol or Propanolol. Antagonists were added 30 minutes before the addition of agonists. (11)

β₂-adrenoceptor assay

Rat uterine horns in estrus phase from female Wistar rats were mounted in organ baths (INCO, Ambala, India) filled with 25 ml of Locke ringer solution of composition (in mM) NaCl 154, KCl 5.6, NaHCO₃ 6.0, CaCl₂ 2.2, glucose 11.1, ascorbic acid 30 μM and sodium salt of EDTA 30 μM. The tissues were maintained at 37 °C and aerated with carbogen. To achieve a steady spontaneous contraction, an initial tension of 2 g was applied for 30 minutes. After the tissues were equilibrated concentration response curves (CRCs) to isoprenaline were constructed by cumulative addition (0.5 log unit increments) to the spontaneously contracting uterus at 3 minutes interval until the uterus completely relaxed. The tissues were washed for 30 minutes with Locke ringer solution and were allowed to equilibrate for 30 minutes in presence of PP-28 or Atenolol or Propanolol. The β₂ adrenoceptor antagonistic activity was determined by constructing CRCs of isoprenaline in presence of PP 28 or Atenolol or Propanolol. (12)

β₃-adrenoceptor assay

Distal colon (2.5 cm in length) of Wistar rats was removed, cleared of from any fecal material and mounted with care taken not to occlude the lumen, in 25 ml organ baths (INCO, Ambala, India) containing Krebs' bicarbonate buffer solution, at 37° C bubbled continuously with 95% oxygen under an initial tension of 1g. Additionally, Krebs solution 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 adrenoceptors. Tissues were allowed to equilibrate for atleast 30 min before experimental procedures were started. Relaxant action of agonists was determined by measuring relaxation of KCl induced contraction evoked by addition of the agonists. Initially 50 mM KCl was added after equilibration period. KCl induced contractions were allowed to stabilize for 15 minutes followed by 30 minutes wash. Tissues were again contracted with a submaximal contraction of KCl (30 mM) and washed for 30 minutes. Tissues were then incubated with appropriate concentrations of antagonist for 30 minutes with control tissues receiving saline treatment. The tissues were then contracted again with KCl (30 mM) and allowed to stabilize. CRCs to isoprenaline were constructed by cumulative addition (0.5 log unit increments) to KCl –contracted tissue at 2 minute interval until the tissue relaxes and reaches plateau. (13)

In vivo studies

Evaluation of β adrenoceptor antagonist activity in vivo

Dose response curve to isoprenaline was constructed for increase in heart rate (tachycardia) and fall in Mean arterial pressure (MAP) after intravenous injection of 0.3, 1 and 3 μg/kg of isoprenaline. Next, a single dose of test drug was administered intravenously. Five minutes later, further injection of isoprenaline (0.3, 1 and 3 μg/kg, i.v) was given and the changes in heart rate and MAP were measured. [11]

LRA Ligated hypertension

Male Wistar rats (175-200 g) were anesthetized with Ketamine (50 mg/kg, i.p.) and Xylaxine (10 mg/kg, i.p.) and the left renal artery (LRA) was ligated [14] except in Group I rats. Group I served as normal control animals, which had not, undergone renal ligation. After renal ligation the animals were housed and provided with 1% sodium chloride solution instead of water. After 6 weeks of LRA ligation the animals were divided into 7 groups consisting of 6 animals in each group. Group II received vehicle [0.05 % Tween-80 + 0.5 % CMC, 1 ml/kg, orally.], which served as control. Group III, IV and V received PP-28 (1, 3 and 10 mg/kg) orally respectively. Group VI and VII received standard drug propranolol (30 mg/kg orally) and atenolol (10 mg/kg orally) respectively. Vehicle, PP-28 and drug treatment was initiated after 6 weeks of LRA ligation and treatment was continued for 7 days. LRA ligated rats were found to be hypertensive after 6 weeks when mean arterial pressure (MAP) was recorded by directly cannulating the carotid artery. At the end of 7th week, after 1 hour of administration of last dose of saline or test drugs MAP and Heart rate was recorded according to given procedure.

Fructose induce hypertension

Forty two male Wistar rat (210-230 g) were randomly divided into eight group (n=6 in each group). Normol control group were given ordinary drinking water ad libitum throughout the whole treatment course and the remaining group were given 10% fructose solutions to drink ad libitum [15]. Nine weeks later, the fructose-treated animals were assigned the following treatment regimen; Group II received vehicle [0.05 % Tween-80 + 0.5 % CMC, 1 ml/kg, orally.] which served as fructose fed control group control. Group III, IV, and V received PP-28 (1, 3 and 10 mg/kg, orally) orally respectively. Group VI and VII received standard drug propranolol (30 mg/kg orally) and atenolol (10 mg/kg orally) Saline or drug treatment was initiated after 9 weeks of fructose feeding and treatment was continued for 7 days. At the end of 7th day, after 1 hour of administration of last dose of saline or test drugs MAP and Heart rate was recorded according to given procedure.

Measurement of Mean arterial pressure and heart rate

Wistar rats were anaesthetized with urethane (1.25 g/kg, i.p.). Trachea and carotid artery were cannulated with the help of polyethylene catheter containing heparin dissolved in isotonic saline (100 IU/ml). Body temperature was maintained at 37⁰ 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 MAP and heart rate.

Data Analysis

Data in table and fig. are expressed as mean \pm SEM. Significant differences were determined by the independent and paired student's t- test in unpaired and paired samples, respectively. Whenever a control group was compared with more than one treated groups one-way analysis of variance followed by the Dunnett test used for analysis. Values for $P \leq 0.05$ were considered significant. Analysis of data and plotting were done with the aid of software (InStat[®], Version 3.0 and Graph Pad PRISM[®], Version 4.0, San Diego, CA, USA) run on Windows operating system.

Results

 β -Adrenoceptor blockade and selectivity

Table 1 displays pA_2 values of PP-28, atenolol and propranolol treatment, calculated from Schild plots. In all cases, the slopes of Schild plots were not significantly different from 1.0. PP-28 antagonized the isoproterenol induced positive chronotropic effects on the isolated wistar rat right atrial strips. In addition, PP-28 caused a dose-dependent parallel shift to the right of the isoproterenol concentration response curves, yielding pA_2 values of 7.29 ± 0.17 (Table-1).

PP-28 antagonized isoprenaline induced relaxation of spontaneously contracting uterus and KCl induced contraction in rat colon. PP-28 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 adrenoceptor antagonistic activity. The apparent pA_2 values of PP-28 for β_2 and β_3 adrenoceptor were found to be 5.68 ± 0.09 and 5.94 ± 0.09 respectively.

The β_1/β_2 -selectivity ratio was calculated from the antilogarithm of the difference between the pA_2 values obtained from rat right atrial strips and uterus. The estimated β_1/β_2 -selectivity ratio value (40.73) indicated that PP-28 had high affinity to β_1 adrenoceptor than to β_2 adrenoceptor subtypes. The relative order of β_1/β_2 -selectivity was found to be in the order of PP-28 \geq Atenolol > Propranolol.

Table 1: β Adrenoceptor blocking potency and selectivity of PP-28.

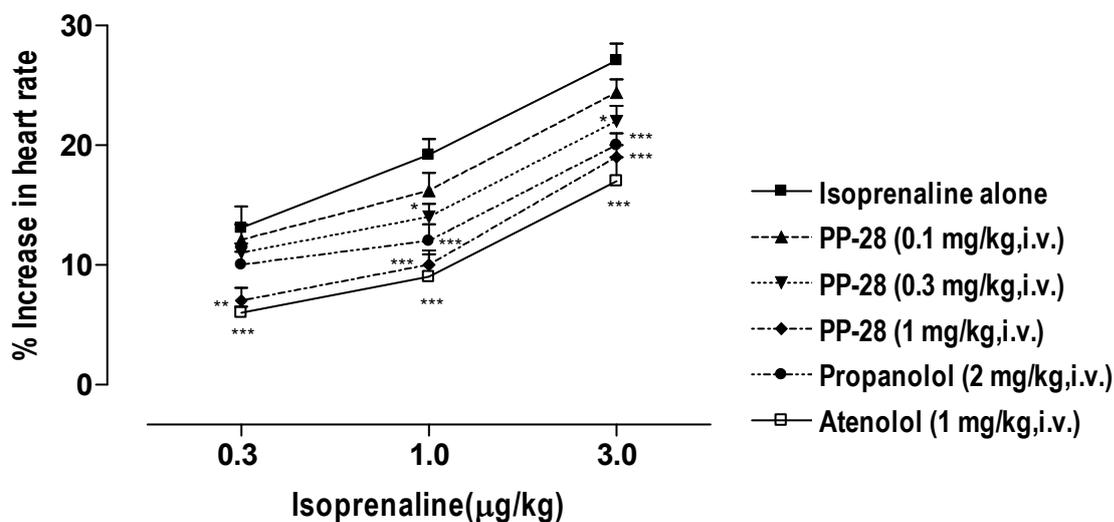
β blocker	pA_2 values			Selectivity ratio	
	β_1 Right atria (slope)	β_2 Uterus (slope)	β_3 Colon (slope)	β_1/β_2	β_1/β_3
PP-28	7.29 ± 0.17 (1.09 ± 0.59)	5.68 ± 0.09 (1.07 ± 0.17)	5.94 ± 0.09 (0.92 ± 0.16)	40.73	22.38
Propranolol	8.27 ± 0.02 (1.03 ± 0.07)	8.18 ± 0.07 (0.96 ± 0.01)	6.64 ± 0.16 (0.93 ± 0.16)	1.23	42.65
Atenolol	7.15 ± 0.11 (1.18 ± 0.09)	5.56 ± 0.06 (0.99 ± 0.11)	4.61 ± 0.33 (0.94 ± 0.05)	38.90	346.73

The 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 $pA_2 = [\log(DR-1) \times \log \text{ molar concentration antagonist}]$ and the slope values were calculated from individual Schild plot by regression analysis. Each value was the mean \pm S.E.M. of six to eight experimental results.

Hypotensive and bradycardia effects

Administration of isoprenaline (0.3 µg/kg, 1 µg/kg and 3 µg/kg, i.v.) produced a dose dependent increase in heart rate (tachycardia) and decrease in the MAP. Intravenous administration of PP-28 (0.3, 1 and 3 mg/kg, i.v.), propranolol (3 mg/kg) and atenolol (1 mg/kg) alone produced hypotension and reduction in heart rate. Propranolol caused a temporary increase in MAP followed by decrease in blood pressure. The bradycardia effects of these compounds could be placed in the following order PP-28 ≥ atenolol > propranolol as shown in figure 2. Administration of isoprenaline (0.3 µg/kg, 1 µg/kg and 3 µg/kg, i.v.) in the presence of PP-28 (0.3, 1 and 3 mg/kg, i.v.), propranolol (3 mg/kg, i.v.) and atenolol (1 mg/kg, i.v.) significantly reduced isoprenaline induced tachycardia and hypotension. Inhibition of cardioaccelerator and hypotensive responses to isoprenaline by PP-28 revealed β adrenoreceptor blocking activity.

A.



B.

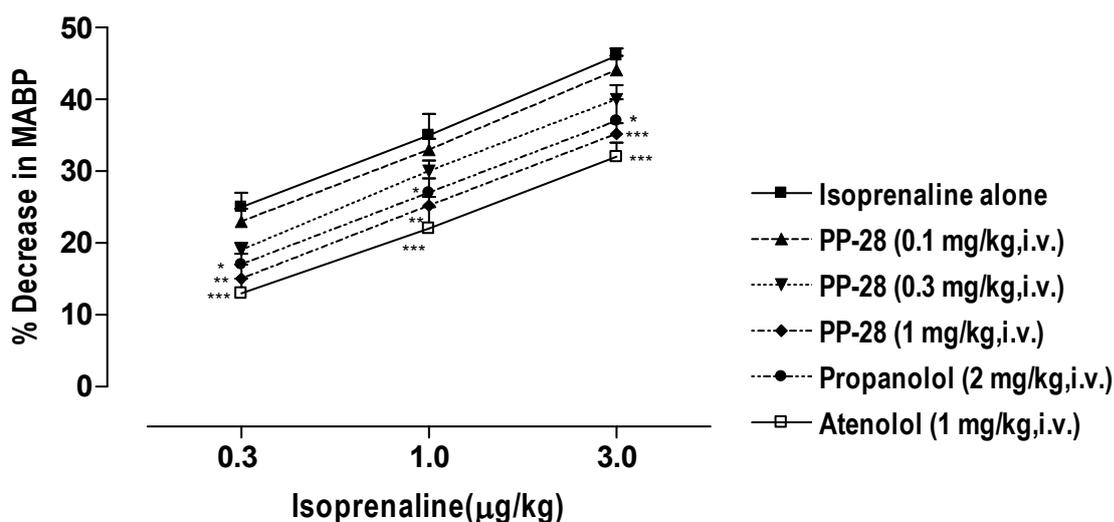
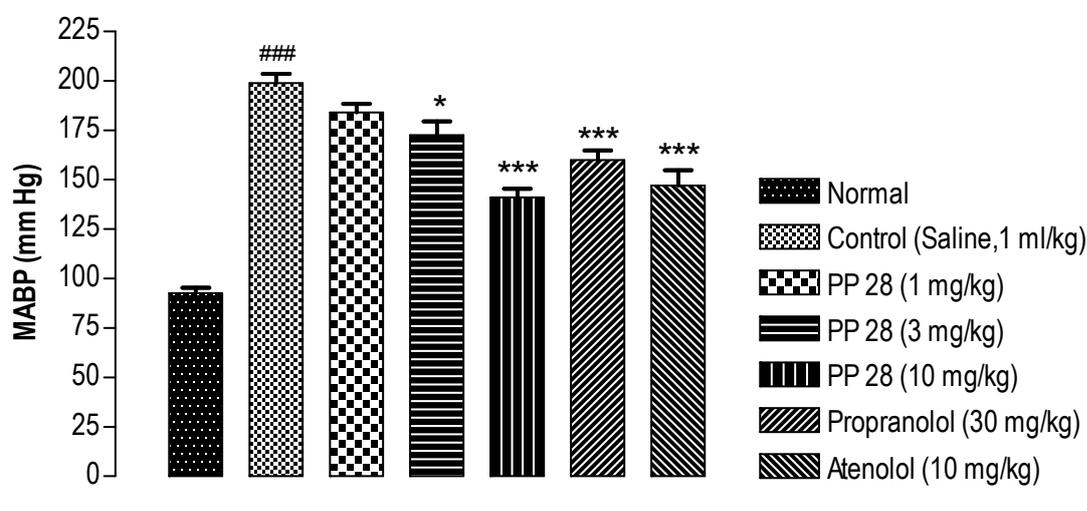


Figure 2: % Increase in heart rate (A) and % decrease in mean arterial blood pressure in normol rats with Isoprenaline alone or with PP-28, Atenolol and Propranolol. N = 6 rats per group. Data are expressed as mean ± SEM. Two-way analysis of variance followed by Bonferroni post tests. ###P <0.001, #P <0.05 as compared to normal group, *P <0.05, **P <0.01, ***P <0.001 as compared to control group.

*In vivo assessment of antihypertensive activity**LRA Ligated hypertension*

The mean arterial blood pressure significantly increases ($^{###}P < 0.001$) in left renal artery ligated control group when compare with normal group. Treatment with PP 28 (1, 3 and 10 mg/kg,) produced a dose dependent decrease in MAP and heart rate of LRA ligated rats. Significant reduction in MAP and heart rate was observed in hypertensive rats treated with PP 28 (3 and 10 mg/kg), propranolol (30 mg/kg) and atenolol (10 mg/kg). The fall in MAP produced by PP 28 (10 mg/kg) was similar with atenolol (10 mg/kg) and greater than propranolol (30 mg/kg i.p.) are shown in Figure 3.

A.



B.

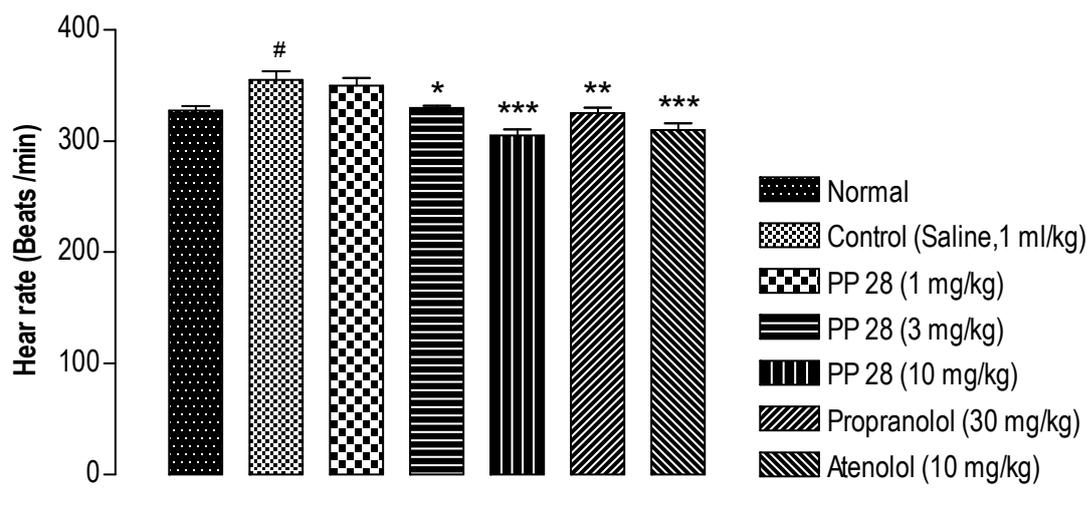


Figure 3: Mean arterial blood pressure (A) and heart rate (B) of LRA Ligated rats drinking ordinary tap water or 1% NaCl after 7 days treatment with PP-28, Atenolol and propranolol. N = 6 rats per group. Data are expressed as mean \pm SEM. One-way analysis of variance followed by $^{###}P < 0.001$, $^{\#}P < 0.05$ as compared to normal group, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to control group.

Fructose induce hypertension

The effect of 10 % fructose, PP-28, atenolol and propranolol on MAP and heart rate are shown in Figure 4. Fructose treatment was associated with a significant increase ($^{###}P < 0.001$) in MAP and ($^{\#}P < 0.05$) in heart rate when compare with control group. Treatment with PP 28 (1, 3 and 10 mg/kg,) produced a dose dependent decrease in MAP and heart rate of fructose induce hypertensive rat. Significant reduction in MAP and heart rate was observed in hypertensive rats treated with PP 28 (10 mg/kg), propranolol (30 mg/kg) and atenolol (10 mg/kg). The fall in MAP produced by PP 28 (10 mg/kg) was less than atenolol (10 mg/kg) and equivalent to propranolol (30 mg/kg i.p.).

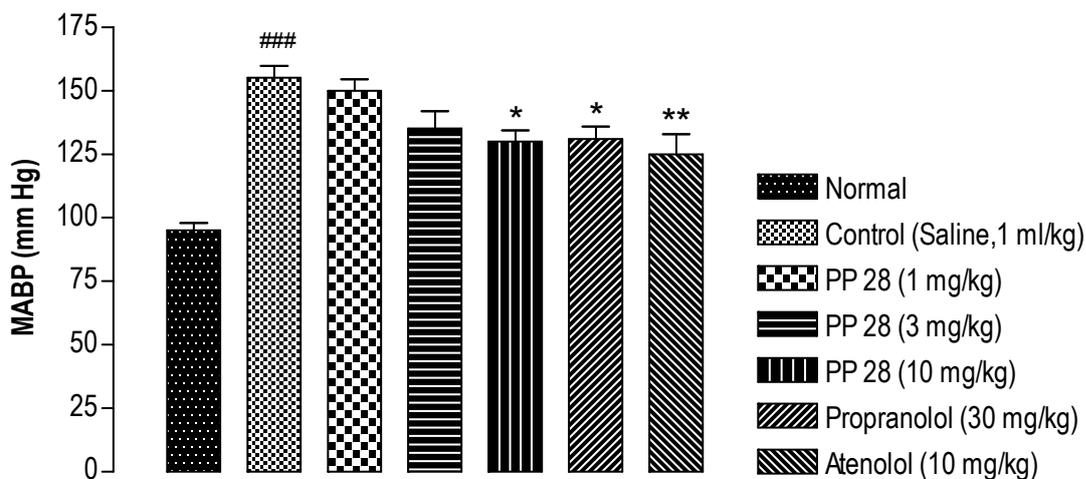
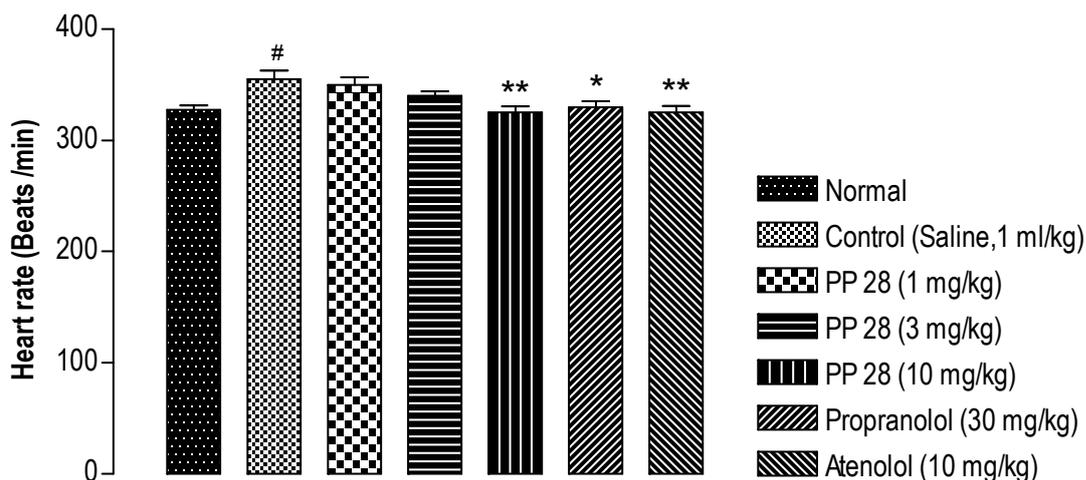
A.**B.**

Figure 4: Mean arterial blood pressure (A) and heart rate (B) of rats drinking ordinary tap water or 10% fructose after 7 days treatment with PP-28, Atenolol and propranolol. N = 6 rats per group. Data are expressed as mean \pm SEM. One-way analysis of variance followed by Tukey's Multiple Comparison Test. $^{###}P < 0.001$, $^{\#}P < 0.05$ as compared to normal group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to control group.

Discussion

Isoprenaline is a potent non-selective β -adrenoceptors agonist with low affinity for α adrenoceptors. Consequently isoprenaline has powerful effects on all β -adrenoceptors and no action on α adrenoceptors. A number of investigators have reported that β_1 -adrenoceptors preferentially exists in human and various rodent hearts. β_1 -adrenoceptors mediates positive inotropic and chronotropic effects of isoprenaline in the heart (16).

PP-28, atenolol and propranolol antagonized competitively the beating rate brought by the isoprenaline, suggesting that PP-28 possess beta1 adrenoceptor antagonistic activity. The rank order potency for β_1 adrenoceptor was found to be PP-28 \geq Atenolol $>$ Propranolol. Isoprenaline inhibit the uterine smooth muscle contraction, which is mediated through β -adrenoceptors. The presence of vast majority of β_2 -adrenoceptors in rat uterus was confirmed by autoradiography localization and the β_2 -adrenoceptors mediated response was influenced by the length of gestation (12). In the present experiment, administration of isoprenaline abolished spontaneous contraction of uterus. PP-28, propranolol and atenolol shifted the concentration response curve towards right indicating β_2 -adrenoceptor antagonistic activity. The presence of β_3 -adrenoceptor has been confirmed in human and rodent colon (17). In the present investigation, relaxation to isoprenaline of KCl induced contraction in rat colon was inhibited by PP-28 suggesting that it possess β_3 antagonistic activity. The β_1/β_2 -selectivity ratio for PP-28, atenolol and propranolol were 40.73, 38.90 and 1.23 respectively. These results suggest that PP-28 and atenolol is a highly selective β_1 blocker. Hence the risks like bronchoconstriction in asthmatics can be reduced. The relative order of β_1/β_2 -selectivity was found to be in the order of PP-28 \geq Atenolol $>$ Propranolol.

Isoprenaline is a potent β -adrenoceptor agonist with high affinity for all β adrenoceptors, devoid of any action on α adrenoceptors. Intravenous infusion of isoprenaline stimulates cardiac β adrenoceptors and lowers peripheral vascular resistance. The tachycardia produced by isoprenaline is primarily due to the β -adrenoceptors activity in heart and the hypotensive effect is primarily due to the β_2 adrenoceptors in the blood vessels (18). β_1 , β_2 and β_3 adrenoceptors are present in mammalian heart. Positive inotropic and chronotropic effect of isoprenaline *in vivo* is brought through β_1 adrenoceptors. Further, catecholamine induced positive chronotropic and inotropic effects were completely absent in β_1 adrenoceptors deficient mice (19). In contrast, deletion of the β_2 adrenoceptor gene did not alter cardiac responses to catecholamine (20). These observations suggest that isoprenaline induced tachycardia is primarily mediated through β_1 adrenoceptors in heart. In our study, administration of isoprenaline produced tachycardia and hypotension in anesthetized rats. The percentage increase in heart rate and hypotension by isoprenaline (3 $\mu\text{g}/\text{kg}$, i.v.) are well in correlation with earlier reports. Isoprenaline induced tachycardia was blocked by PP-28 (0.3 mg/kg, i.v.) and atenolol (1 mg/kg, i.v.) while the hypotensive effect of isoprenaline was not blocked at this dose levels implying selective blockade of cardiac β -adrenoceptors (β_1) at this dose levels. However, PP-28 (1 mg/kg, i.v.), Atenolol (1 mg/kg, i.v.) and propranolol (2 mg/kg) blocked both tachycardial and hypotensive effect of isoprenaline, suggesting blockade of β_1 and β_2 adrenoceptors in heart and blood vessels by these drugs at the above dose levels. The *in vivo* experiments suggested that PP-28 possess cardio selective β -blockade at lower dose levels. However the selectivity was relative and lost at higher doses.

The mechanism involved in the development of hypertension in LRA ligated model appears to depend on the species Examined. In rat, constriction of renal artery with an intact contralateral kidney produces hypertension with an elevated plasma rennin activity (PRA) and the animals are responsive to angiotensin blockade [14]. β -blockers decreases blood pressure by

different mechanism, reduction in the cardiac out put, reduction of rennin 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 [14]. In the present investigation PP-28 showed significant anti hypertensive effect. The antihypertensive effect possessed by PP-28 was greater than the atenolol and propranolol. The greater potency of PP-28 in LRA ligated hypertensive rats is may be due to its stronger receptor (β_1) binding property compared to atenolol and propranolol.

Several anti-hypertensive drugs effectively prevent and reverse the increase in blood pressure induced by high fructose diets [21, 22 and 23]. The results of this study demonstrate that 9 weeks of high fructose feeding in rats resulted in a hypertensive state that was normalized by treatment with the PP-28, atenolol and propranolol. The PP-28 reversed the increase in SBP in a dose-dependent manner. The results of our study confirm that fructose feeding can induce hypertension in normal Wistar rats. It is known that high-fructose feeding leads to hypertension by Several mechanisms including sodium retention and fluid volume expansion [24, 25]. Stimulation of the sympathetic nervous system and vascular small muscle proliferation [26], increase in cytosolic [Ca^{2+}] ion [27]. The PP-28 like, atenolol and propranolol in our findings provoked a decrease of the higher MAP induced by fructose in one weak treatment in a concentration-dependent manner. The PP-28 also reduced the extent of development of hypertension induced by the high fructose-diet. The results of this study cannot explain the precise mechanism of action of PP-28. Yet these results suggest that PP-28 can play a major role in the mechanisms underlying the pathogenesis of fructose-fed hypertension as demonstrated by the beneficial effects on blood pressure.

Conclusion

In conclusion, PP-28 possessed strong affinity to β_1 adrenoreceptor subtypes. This study has shown the antihypertensive effect of PP-28 the using the LRA ligated rats and fructose drinking rat model. These results may lend further support to mount up evidence that the PP-28, if taken in sufficient quantities, could conceivably be beneficial in the attenuation and prevention of hypertension. Further studies are required to establish the mechanism underlying the antihypertensive effect of PP-28.

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