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Synthesis, Spasmolytic, Cardio-suppressant, Vasodilator and Ca^{++} Antagonist Activities of 1-[4′-methylphenacyl]-4-acetyl -4-phenylpiperidinium bromide

[1,4]S.I.H. Taqvi, [2]M.N. Ghayur, [2]A.H. Gilani, [3]M.T. Aftab and [4]Z.S. Saify
[1]Federal Urdu University of Arts, Science and Technology, University Road, Karachi, Pakistan
[2]Department of Biomedical and Biological Sciences, The Aga Khan University, Stadium Road, Karachi-74800, Pakistan
[3]Post Graduate Medical Institute, Baqai Medical University, Karachi, Pakistan
[4]Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

Abstract: The smooth muscle relaxant and cardiovascular suppressant activities of a newly synthesized piperidine derivative: (1-[4′-methylphenacyl]-4-acetyl-4-phenylpiperidinium bromide) were studied in isolated tissue preparations. The test compound exhibited dose-dependent relaxant effect on the spontaneous and K^+ (75 mM)-induced contractions of isolated rabbit jejunum with respective EC_{50} values of 0.056 mM (0.011-0.289, 95% CI) and 0.052 mM (0.004-0.769). The Ca^{++} channel blocking (CCB) activity was confirmed when the test compound (0.005-0.020 mM) shifted the Ca^{++} dose-response curves to the right, similar to that produced by verapamil (0.3-1.0 μM), a standard CCB. In the isolated rabbit aorta, the test compound showed a dose-dependent vasodilator effect on norepinephrine (1 μM) or K^+ (75 mM)-induced contractions with an EC_{50} value of 0.016 mM (0.005-0.048). When tested in Langendorff perfused rabbit heart preparation, it exhibited a negative chronotropic effect in atria and ventricles with respective EC_{50} values of 0.79 mM (0.45-2.05) and 0.63 mM (0.32-1.52) and also a negative inotropic effect in atria and ventricles with respective EC_{50} values of 0.98 mM (0.30-3.19) and 8.98 mM (5.98-13.50). The results showed that inhibitory effects of the piperidine derivative on intestinal and cardiovascular preparations are mediated possibly via blockage of voltage and receptor-operated Ca^{++} channels.

Key words: Piperidine analogue, spasmolytic, Ca^{++} antagonist, vasodilator, cardio-suppressant

INTRODUCTION

Piperidine analogues are well known as analgesics and spasmolytics albeit to date very few piperidine derivatives have been reported which are cardio-active and calcium channel blockers (CCB) as well. However, a few studies have been reported in the literature, such as selective Ca^{++} antagonist activity of some new piperidine analogues in the myocardium of guinea pig and dog[1]; suppressing effect of bupavacine on [Ca^{++}] oscillations in neonatal rat myocaridocytes[2]; CCB effect of fentanil, sufentanil and remifentanil[3]; Na^+ and Ca^{++} channel blocking activities of 4-arylpiperidine and 4-arylpiperidinols[4]; negative inotropic effect of meperidine via L-type CCB in rat heart preparations[5] and CCB activity of N-n-butylhaloperidol iodide in rat myocardial ischemia and reperfusion injury[6].

In view of the therapeutic potential, different piperidine analogues have been synthesized and studied for their vasodilator and hypotensive effects[7-9]. In this study we report the intestinal spasmolytic, cardio-suppressant, vasodilator and Ca^{++} antagonist activities of 1-[4'-methylphenacyl]-4-acetyl-4-phenylpiperidinium bromide (Fig. 1), a new piperidine analogue chemically belonging to phenylpiperidine group of piperidine nucleus-containing compounds[10].

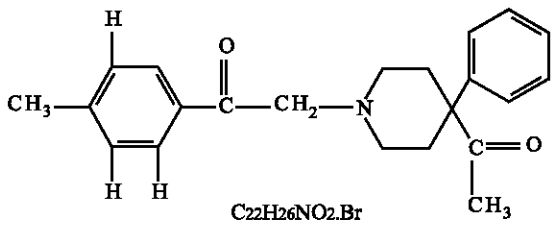

Fig. 1: Chemical structure and formula of the test compound1-[4′-methylphenacyl]-4-acetyl-4-phenylpiperidinium bromide

Corresponding Author: Dr. Anwarul Hassan Gilani, Professor of Pharmacology, Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi-74800, Pakistan
Tel: +92 21 486 4571 Fax: 92 21 493 4294,2095

MATERIALS AND METHODS

Synthesis of the test compound: Equimolar quantity of 4-acetyl-4-phenylpiperidine and 1-(4-methylphenacyl) bromide were dissolved in acetone and refluxed on a water bath and the reaction was continuously monitored by TLC using the solvent system $CHCl_3$-MeOH in the ratio of 9:1.When all the starting material changed into product, the resulting solid material or the precipitate was collected by filtration and thoroughly washed to remove traces of reactant. It was then dissolved and recrystallized from ethyl alcohol: yield 58%, m.p. 226-228°C.

Spectral Data:
U.V. (MeOH) 1_{max} nm: 258, 203
I.R. (KBr) n/cm: 3400, 2900, 2650, 1680, 1340
E. I. M. S. m/z (%) 335(M^+-HBr, $C_{22}H_{25}NO_51$), 216(100), 203(1), 173(6), 103(5), 115(4), 129(5), 82(13)
1H-N. M. R. (CD_3OD) 8=7.91(2H, d, J=8.31 Hz, 2', 6'-H), 7.48(2H, d, J=8.31 Hz, 3', 5'-H), 7.37(5H, m, 2", 6"-H), 6.32(2H, s, á-H), 2.89-2.65(4H, m, 2, 6-H), 2.04-1.90(4H, m, 3,5-H), 2.43(3H, s, 4'-CH_3), 1.98(3H, s, $COCH_3$), $C_{22}H_{26}BrNO_2$ Formula Weight: 416.37, Calc 663.46, $H_{6.29}$ Found $C_{63.31}$, $H_{6.21}$, $N_{3.32}$

Drugs and chemicals: Acetylcholine (ACh), norepinephrine (NE) and verapamil were obtained from Sigma Chemical Company, St. Louis, MO, USA while heparin injections BP were purchased from Rotex Medica, Trittau, Germany. The following chemicals were used to make the physiological salt solutions: potassium chloride (Sigma Chemical Company, St. Louis, MO, U.S.A), calcium chloride, glucose, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, sodium dihydrogen phosphate (E. Merck, Darmstadt, Germany) and ethylenediaminetetra-acetic acid (EDTA) from BDH Laboratory Supplies, Poole, England. Stock solutions of all the chemicals were made in saline fresh on the day of the experiment.

Animals: Experiments performed compiled with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council[11]. Local male rabbits (around 1 kg) used in the study were housed in the animal house of the Aga Khan University under a controlled environment (23-25°C). Animals were given tap water *ad libitum* and a standard diet consisting of (g kg^{-1}): flour 380, fibre 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium metabisulphate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

Isolated rabbit jejunum: Experiments were performed as described earlier[12]. Segments of rabbit jejunum tissue 2 cm long were suspended in 10 mL tissue baths containing Tyrode's solution, aerated with a mixture of 95% oxygen and 5% carbon dioxide (carbogen) and maintained at 37°C. The composition of Tyrode's solution in mM was: KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8 and glucose 5.55. Intestinal responses were recorded isotonically using Harvard student oscillographs and isotonic transducers. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Under these conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing of relaxant (spasmolytic) activity directly with out the use of an agonist.

Determination of Ca^{++} antagonist activity in rabbit jejunum: To assess whether the spasmolytic activity of the test compound was mediated through CCB, K^+ (75 mM) was used to depolarize the preparations[13]. High K^+ (75 mM) was added to the tissue bath, which produced a sustained contraction. The test compound was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses[14]. The relaxation of intestinal preparations, precontracted with K^+ (75 mM) was expressed as percent of the control response mediated by K^+. Contraction of smooth muscle induced by K^+ is known to be mediated, via influx of Ca^{++} from extracellular fluid and a substance, which inhibits this contraction, is considered to act through blockade of Ca^{++} channels[15].

To confirm the Ca^{++} antagonist activity of the test compound, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{++}-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove Ca^{++} from the tissues. This solution was further replaced with K^+-rich and Ca^{++}-free Tyrode's solution, having the following composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1. Following an incubation period of 30 min, control dose-response curves (DRCs) of Ca^{++} were obtained. When the control DRCs of Ca^{++} were found super-imposable (usually after two cycles), the tissue was pretreated with the test compound for 60 min to test the possible CCB effect. The DRCs of Ca^{++} were reconstructed in the presence of different concentrations of the test compound while verapamil was used as a positive control.

Isolated rabbit aorta: Rabbits were sacrificed by cervical dislocation. The descending thoracic aorta was

removed and cut into 2-3 mm wide rings which were individually mounted in 20 ml tissue baths containing Kreb's-Henseliet solution (composition in mM: NaCl 11.50, KCl 4.70, $CaCl_2$ 2.50, $NaHCO_3$ 25.0, $MgSO_4.7H_2O$ 1.50, $K_2H_2PO_4$ $2H_2O$ 1.20 and glucose 11.0) at 37°C and aerated with carbogen gas. A resting tension of 2 g was applied to each tissue and an equilibrium period of 1 h was allowed before any experimentation. The changes in isometric tensions of the rings were measured via a force-displacement transducer (FT-03) using a Grass Model 7 Polygraph. Following an equilibrium period of 1 h, the tissues were stabilized with a fixed dose of NE (1 μM). The tissues were considered stable only when similar responses were obtained from the repeated doses of NE (1 μM). Effect of the test compound was first determined on the resting baseline of the tissue to see if it has any vasoconstrictor effect. Later it was tested for any ability to relax the high K^+ (75 mM)-induced contractions or control NE (1 μM) peak responses. The ability of the extract to relax K^+ (80 mM)-induced contractions would indicate L-type voltage-dependent CCB mode of vasodilation while inhibition of the NE-peak responses would signify the blockade of the Ca^{++} influx through the receptor-operated Ca^{++} channels[16]. Procedure for the latter possibility involved incubating the control NE responses with increasing doses (0.05-0.5 mM) of the test compound for 1 h.

Langendorff perfused rabbit heart: Whole hearts were obtained from healthy rabbits (male, 1 kg). Heparin (5000 I.U) was injected (i.p) 1 h prior to isolation of the whole hearts. After cervical dislocation, hearts were excised rapidly and mounted on Langendorff apparatus as quickly as possible. Kreb's-Henseliet solution perfused the heart retrogradely, aerated by carbogen at thermostatically controlled temperature (37°C) with pH of 7.4. Atrial and ventricular activities were recorded simultaneously and separately by two different Harvard isotonic transducers. Approximately 60 min were allowed to each heart to adapt to the new environment and to exhibit sino-atrial nodal pattern of the cardiac activity. Any heart showing an abnormal pattern was discarded. After taking 10 min of equilibrium period, the test compound was added in ascending order. For each dose, 10 min were allowed to achieve the peak effect. Changes in atrial and ventricular activity were calculated when maximal effect persisted for 5 min or more[17].

Statistical analysis: All the data expressed are mean±standard error of mean (SEM, n = number of experiments). The statistical parameter applied is the paired Student's t-test with $p<0.05$ noted as significantly different (GraphPAD program, GraphPAD, San Diego, CA, USA). Concentration-response curves were analyzed by non-linear regression (GraphPAD program).

RESULTS

Effect on rabbit jejunum: When tested on the spontaneously contracting rabbit jejunum, the test compound caused a dose-dependent (0.02-0.5 mM) relaxant activity (Fig. 2) with an EC_{50} value of 0.056 mM (0.011-0.289, 95% CI, n=5). When tested on the high K^+ (75 mM)-induced contractions, the test compound exhibited a dose-dependent (0.01-0.1 mM) inhibition (Fig. 2) with an EC_{50} value of 0.052 mM (0.004-0.769, n=5). The interaction with Ca^{++} channels was further studied in jejunum, which is known to be quick in responding to spasmolytic activity[18]. The test compound dose-dependently (0.005-0.02 mM, n=3) shifted the Ca^{++} dose-response curves to the right (Fig. 3A), similar to that produced by verapamil (0.3-1.0 μM, n=3; Fig. 3B).

Effect on rabbit aorta: The test compound was found devoid of any contractile activity on the resting baseline however, when tested on high K^+ (75 mM)-induced contractions, it showed a dose-dependent (0.001-0.5 mM) vasodilator effect (Fig. 4A) with EC_{50} value of 0.016 mM (0.005-0.048, n=5). Likewise, the test compound dose-dependently (0.05-0.5 mM) inhibited the control peak responses of NE (1 μM) after pretreating the peaks with each of the test compound dose for 1 h (Fig. 4B).

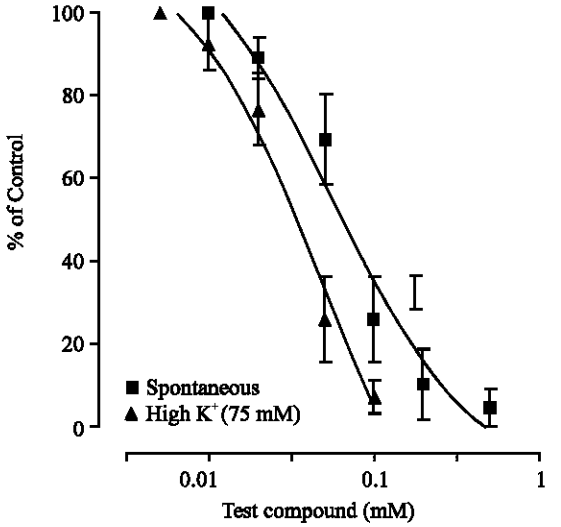

Fig. 2: Dose-response curves showing the dose-dependent spasmolytic effect of the test compound in spontaneous and high K^+ (75 mM)-contracted isolated rabbit jejunum (values shown are mean ±SEM, n=5)

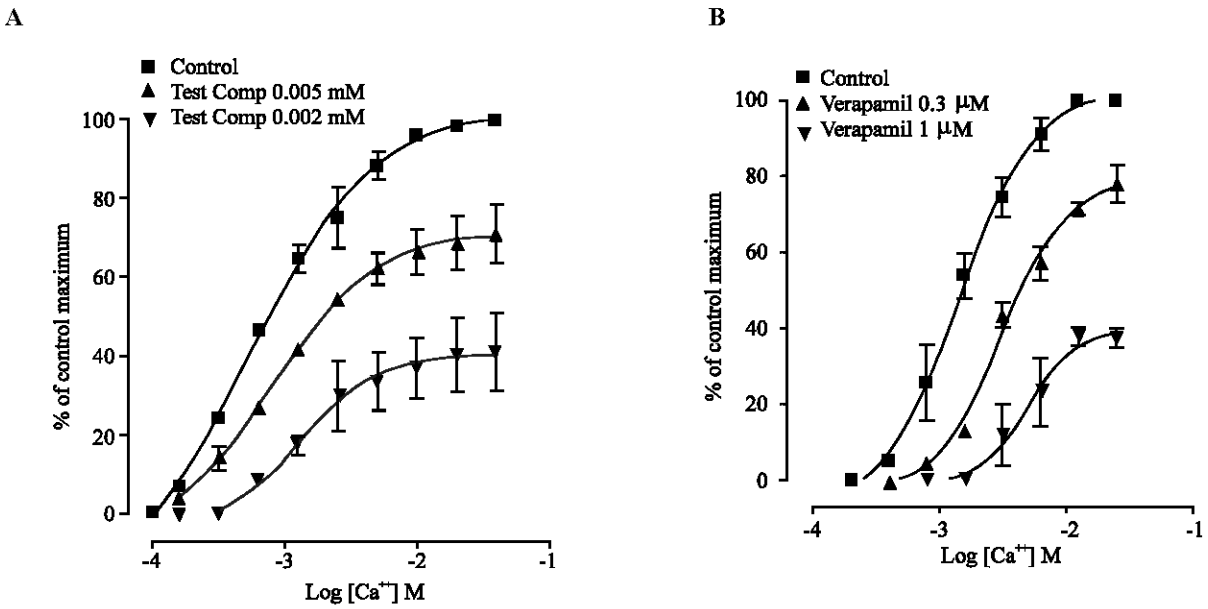

Fig. 3: Dose-response curves showing the inhibitory effect of increasing doses of [A] test compound and [B] verapamil on Ca^{++} concentration-response curves, constructed in a Ca^{++}-free medium, in rabbit jejunum preparations (values shown are mean ± SEM, n=3)

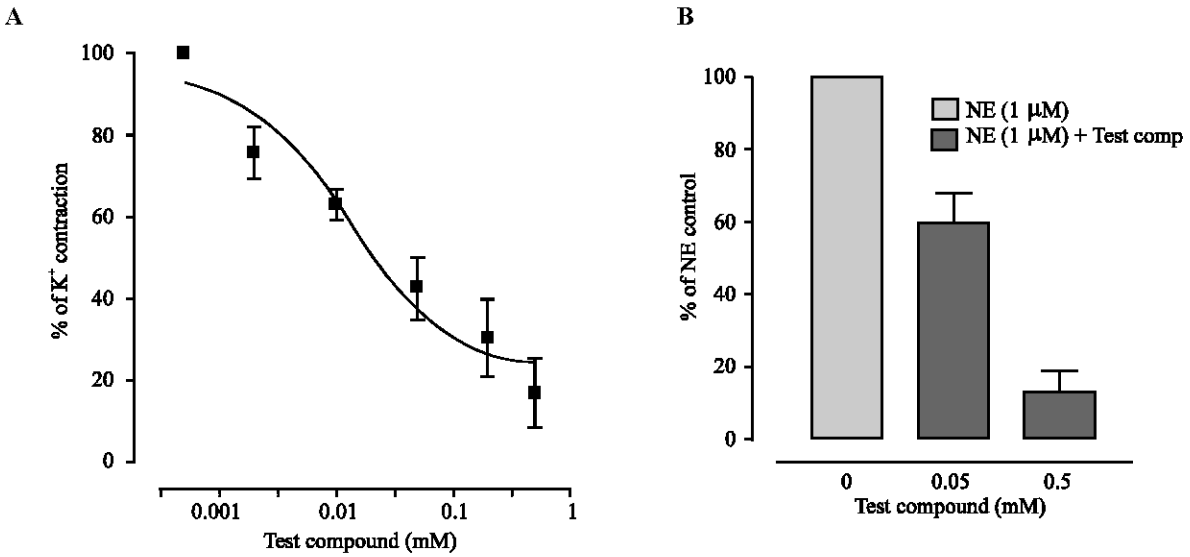

Fig. 4: Inhibitory effect of increasing doses of the test compound on [A] high K^+ (75 mM)-induced contractions and [B] norepinephrine (NE, 1 µM) control peak responses in isolated rabbit aorta (values shown are mean ± SEM, n=5)

Effect on Langendorff perfused rabbit heart: The test compound produced a dose-dependent (0.0001-4 mM) negative chronotropic effect (Fig. 5A) in the atrial and ventricular preparations with EC_{50} values of 0.79 mM (0.45-2.05, n=5) and 0.63 mM (0.32-1.52, n=5), respectively. When tested for an effect on the force of atrial and ventricular beating, the test compound exhibited a negative inotropic effect (Fig. 5B) with EC_{50} values of 0.98 mM (0.3-3.190, n=5) and 8.98 mM (5.98-13.50, n=5), respectively.

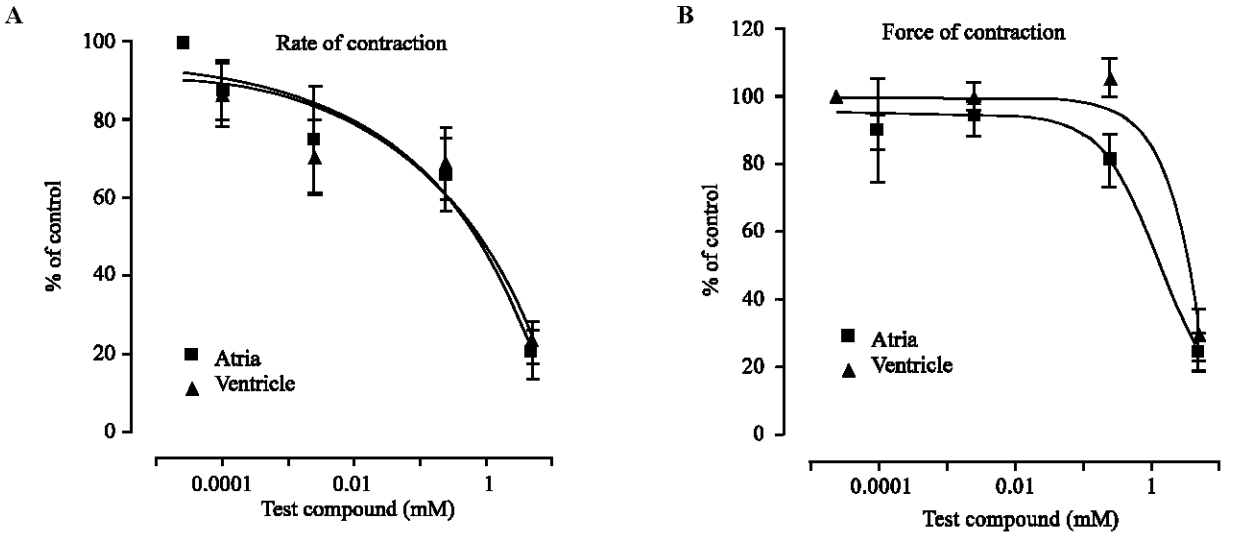

Fig. 5: Dose-response curves showing the inhibitory effect of increasing doses of the test compound on rate and force of atrial and ventricular contraction of rabbit whole heart perfused preparation (values shown are mean±SEM, n=5)

DISCUSSION

The test compound when tested on isolated rabbit jejunum exhibited dose-dependent relaxation of spontaneous and K^+ (75 mM)-induced contractions indicating smooth muscle relaxant activity. The contractions of smooth muscles, including that of rabbit jejunum, are dependent upon an increase in the cytoplasmic free Ca^{++}, which activates the contractile elements[16]. The contraction induced by high K^+ is dependent upon the entry of Ca^{++} into the cells through the voltage-operated channels (VOC) while inhibition of high K^+-induced contraction is due to the result of blocked Ca^{++} entry through these VOCs[15], a characteristic of CCBs. This possible CCB activity of the test compound was later confirmed when it suppressed the Ca^{++} dose-response curves, constructed in a Ca^{++}-free medium, similar to verapamil, a standard CCB[15,19].

In view of the well established use of CCBs in cardiovascular disorders such as hypertension[20-22], the test compound was tested in the isolated aorta and whole heart preparations. The test compound was devoid of any vasoconstrictor activity on the resting baseline of the rabbit aorta but relaxed the high-K^+-induced contraction, dose-dependently, thus reiterating the already observed CCB activity. The test compound was also able to inhibit, in increasing doses, NE (1 μM) peak responses indicating inhibition of receptor-operated Ca^{++} channel (ROCs) as well and thus suggesting non-specific Ca^{++}-antagonist activity of the test compound[23].

In Langendorff perfused rabbit heart, the test compound induced a dose-dependent negative chronotropic and inotropic effect. The compound was equipotent ($p>0.05$) in exhibiting the inhibitory responses on rate and force of contraction in atria and ventricles. The reduction in rate of contraction is known to be due to reduction in trans-sarcolemmal Ca^{++} influx[24] while reduction in force of contraction is the result of inhibition of transmembrane Ca^{++} influx through L-type Ca^{++} channels[25,26]. The doses at which the test compound exhibited the cardio-suppressant actions, seems to be high. This could be firstly due to the limitation of the technique used as large doses of a test substance are needed keeping in mind the degree of dilution done to the test substance in the perfusion liquid and secondly due to the fact that cardiovascular actions of piperidine nucleus-containing compounds are limited at clinically used doses[27]. Therefore, the use of larger doses of the test compound seems to be rational. The cardiovascular activity of the test compound is consistent with the earlier findings of other piperidine derivatives with vasodilator and hypotensive activities[28,29].

The test compound showed intestinal spasmolytic along with cardio-suppressant and vasodilator activities mediated possibly through Calcium Channels blockage.

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