IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 10 Ver. VI (October. 2016), PP 111-117
www.iosrjournals.org

Comparative Myocardial Depressant Activity of Dihydropyrimidine Derivatives 5-Acyl-6-Methyl-4-Phenyl-2-S-Ethyl-1,4-Dihydropyrimidine (BK VI) , 5-Acyl-6-Methyl-4(2,3 Methylenedioxy) Phenyl 2-S-Benzyl-1,4-Dihydropyrimidine (BK VII) And Nifedipine on Isolated Rabbit's Heart.

Poonam Salwan[1], Shalini Salwan[2]
[1]*Assistant Professor,Department of Pharmacology,SGT Medical College And Hospital,Budhera,Gurgaon.*
[2]*Associate Professor ,Department Of Pharmacology ,PIMS, Garha Road Jalandhar.*

Abstract:
Objective:
To investigate the myocardial depressant activity of a dihydropyrimidine derivative 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine (BK-VI) and 5-acyl-6-methyl-4(2,3 methylenedioxy) Phenyl 2-S-benzyl-1,4-dihydropyrimidine (BK VII)and comparing with nifedipine on Isolated perfused rabbit heart.
Material and Methods: *Effects of the test compoundS BK-VI and BK-VII on the amplitude,heart rate and coronary flow on isolated perfused rabbit heart was noted and compared with nifedipine.Observations were made with increasing concentrations of BK-VI, BK-VII and Nifedipine with dimethylsulfoxide(DMSO) as the solvent.Six preparations were used for each dose of BK-VI,BK-VII and nifedipine.Heart rate ,amplitude and coronary flow were noted down every minute for five minutes after injection of each drug and control.*
Results: *On comparing the effects of BK-VI,BK-VII and nifedipine, it was found that all the compounds under investigation had a negative inotropic and negative chronotropic effect. Compound BK -VI did not produce significant effect on heart rate at all the four doses. In comparison, nifedipine produced a significant to highly significant decrease in heart rate.Significant decrease in amplitude with BK-VI was seen at dose of 7.29 X 10^{-4}M and 14.5 X 10^{-4}M by 42% and 56.98% respectively and in coronary flow by 39.95% at dose of 14.5 X 10^{-4}M BK-VII caused significant decrease in heart rate at doses of 26.3 X 10^{-5}M and 52.6 X 10^{-5}M by 13.79% and 43.55% respectively, when compared with the baseline. Significant decrease in amplitude and coronary flow with BK-VII was seen at dose of 52.6 X 10^{-5}M by 84.93% and 52.79% respectively. Nifedipine significantly decreased heart rate and amplitude at all doses with cardiac arrest at a dose of 4.6 X 10^{-5}M. A significant increase in coronary flow was seen with nifedipine by 9.4% and 13% at doses of 0.5 X 10^{-5}M and 1.15 X 10^{-5}M respectively, though this property was lost at higher doses. On comparison, BK-VI and BK-VII produced significant myocardial depression at much higher dose than nifedipine. BK-VII produced these effects at a lower dose than BK-VI.*
Conclusion: *Both BK-VI and BK-VII have calcium channel blocking activity like nifedipine and they produced less potent myocardial depression in comparison with nifedipine.*
Keywords*: Calcium channel blockers, Dihydropyrimidines, Dihydropyridines, , Nifedipine, Voltage dependent calcium channels.*

I. Introduction

Screening of new molecules for their ability to bind to a preselected protein target and to modulate a biological pathway in cells is a part of modern new drug discovery[1]. The driving force behind the development of new technologies for rapid parallel and combinatorial synthesis is the demand for diverse compound libraries for screening in drug discovery [2].Bignelli first described the synthesis of 6-methyl-4-substituted phenyl 2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters by condensation of an aldehyde, urea and ethyl acetoacetate[3].DHP(Dihydropyridines) and DHPMs(Dihydropyrimidines) are extensively studied compounds belonging to the class of calcium channel blockers. Many researchers have studied the synthetic routes and biological properties of these compounds. These developments led to the detailed pharmacological evaluation of these compounds [4]. Considering them as valuable substitutes for nifedipine and other 1,4-dihydropyridine drugs, interest has been shown in the design and synthesis of Bignelli-like compounds [5], regarded as aza-analogues of dihydropyridines [6].Out of the diverse range of biological activities shown by dihydropyrimidines, most notable is the cardiovascular activity that compares favourably

with activity shown by structurally related dihydropyridines - amlodipine, nicardipine. A pharmacophore model for DHP/DHPM calcium channel modulators has been proposed which features a boat-like conformation of DHP/DHPM ring, an axially positioned aryl ring and an ester group at C5 oriented cis with respect to C5=C6 double bond [7].

Dihydropyrimidines uniquely designed to establish structural and conformational determinants for DHP receptor occupation were synthesised and studied for calcium channel modulation.The enantiomers of dihydropyrimidines having up - oriented pseudo-axial aryl group(normal DHP boat) elicit calcium channel antagonistic activity [8] .Compounds with a longer chain at N_3 exhibited less potent vasodilating activity while compounds with shorter length have more potent vasodilator activity [9].Some of these compounds cause increase in coronary flow as well as increase in amplitude, these compounds can be useful in conditions like congestive heart failure[10]. In view of a wide range of biological activity associated with 1,4-dihydropyrimidines and a slight modification in structure can result in qualitative as well as quantitative changes in their activity, this study is an effort to develop such compounds with the aim of having improved activity and lesser toxicity compared with other calcium channel blockers in clinical use.

Two of these compounds 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine (BK- VI) and 5-acyl-6-methyl-4(2,3 methylenedioxy) Phenyl 2-S-benzyl-1,4-dihydropyrimidine (BK VII) , have been taken up in the present study to find out their cardiovascular effects and calcium channel blocking activity.

II. Material And Methods

Test Compounds (BK-VI and BK-VII)

Test compounds 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine(BK-VI),(Molecular weight-274) and 5-acyl-6-methyl-4(2,3 methylenedioxy) Phenyl 2-S-benzyl-1,4-dihydropyrimidine (BK-VII) (Molecular weight 380) were obtained from department of chemistry, Punjabi university, Patiala. For BK-VI, a mixture of benzaldehyde (0.01mole,1.06gms.), thiourea (0.01mole,0.76gms),acetyl acetone (0.015 mole,1.5 ml) and concentrated HCl(3-4 drops) in absolute alcohol was irradiated at 30%microwave power level. The tetrahydropyrimidine obtained was separated, dissolved in NaOH solution and to this mixture, diethyl sulfate was added. BK-VII was prepared using 2,3-methylene dioxy benzaldehyde (0.01 mole, 1.5 gms), thiourea (0.01 mole, 0.76 gm), acetylacetone (0.015 mole, 1.5 ml) and concentrated HCl (3-4 drops) in absolute alcohol (10 ml) taken in a borosil beaker (100ml) were irradiated at 30% microwave power level and to the tetrahydropyrimidine obtained, benzyl chloride was added. The solid products separated were confirmed by taking their IR,NMR, UV and mass spectra[11].Both compounds BK-VI and BK-VII were found to be soluble in Dimethylsulfoxide(DMSO).

Drugs and chemicals

Dimethylsulfoxide(DMSO) was used as a solvent for compounds BK-VI, BK-VII and nifedipine. Other chemicals and agents used were of pure analytical grade and obtained from local suppliers.

Animals

Adult healthy rabbits of either sex weighing between 1.5-2.5 kg were used in this study. They were provided uniform environmental conditions and diet. The diet comprised of green leafy vegetables, grass, soaked grams and milk. The care and maintenance of the animals was as per the approved guidelines of the Committee For the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. All the animal procedures were approved by the Animal Ethical Committee of the establishment. Isolated Perfused Rabbit Heart was used for the present study.

III. Procedure

Isolated perfused rabbit heart

Heart rate: The rabbit heart was mounted as per the methods described by Burn (1952) and Perry (1970). The animals were stunned and bled through carotid arteries. The chest was opened and heart along with an inch of ascending aorta was cut and transferred to a petri-dish containing oxygenated Ringer Locke solution at 37°C. The ventricles were squeezed to remove all blood in order to prevent development of thrombi in the vessels.

The heart was mounted in the Langendorff's assembly. The drug solutions were injected through a polyethylene tube inserted into the rubber tube perfusing the heart. The apex of the heart was attached to the Starling's heart liver with the help of a bent pin passed through the apex and connected to a thread. Baseline recordings were taken after giving a stabilisation time of around 15 minutes. The drugs were administered through the

polyethylene tube and each time, it was followed by injection of 0.2 ml of Ringer Locke solution to ensure complete displacement of the drug. The sensitivity was checked every time by administration of adrenaline 2 µg [11, 12]. Also the effect of test compound was compared with vehicle alone and nifedipine as a control.

Heart rate was counted continuously for 5 minutes after the injection of each drug including the vehicle. The rate was noted down every minute in the five minutes and effect observed. Six such experiments were conducted and mean value calculated.

Amplitude: The amplitude was observed continuously for five minutes after injection of each drug including the vehicle. Six such experiments were conducted and mean value calculated.

Coronary flow: After injection of each drug and control, coronary flow was estimated for five minutes with flow rate noted down every minute. Six such experiments were conducted.

IV. Statistics

Mean value and standard error for all parameters were determined separately and put in tables as percentage change. Statistical significance of the difference at various concentrations, before and after was analysed using Student's paired 't' test.

V. Results

Effect on isolated perfused rabbit heart

Effect of the test compounds BK-VI and BK-VII on the amplitude, heart rate and coronary flow was observed and compared with that of nifedipine. Solutions of compound BK-VI, BK-VII and nifedipine were made in Dimethyl sulfoxide (DMSO) in different concentrations, so that a fixed volume (0.2 ml) was given for every dose.

Effect on Heart Rate

Nifedipine alone caused highly significant to very highly significant decrease in heart rate. The mean percentage decrease was 4.31% ($p<0.01$), 20.75% ($p<0.001$) and 25.17% ($p<0.001$) at doses of $0.05x10^{-4}$M, $0.11x10^{-4}$M and $0.23x10^{-4}$M respectively. A complete cardiac arrest was seen with $0.46x10^{-4}$M dose (Table III), "(Fig.**3**)"

In comparison to nifedipine, BK-VI did not show significant effect on heart rate at all the four doses used. (Table I), (Fig.**1**).However,BK VII showed significant decrease in heart rate at high doses. A significant mean percentage decrease in the heart rate of 13.79% ($p<0.05$) and 43.55% ($p<0.05$) was seen at doses of $26.3x10^{-5}$M and $52.6x10^{-5}$M respectively (Table II), "(Fig.2)".

Effect on amplitude of contraction

Nifedipine showed significant decrease in amplitude with all the doses. The mean percentage decrease in amplitude was 17.97% ($p<0.001$), 57.39% ($p<0.001$), 91.08% ($p<0.001$) at doses of $0.05x10^{-4}$M, $0.11x10^{-4}$M and $0.23x10^{-4}$M respectively. A complete cardiac arrest was seen at $0.46x10^{-4}$M dose (Table III)," (Fig.**3**)"

In comparison to Nifedipine, BK-VI showed decrease in amplitude of contraction at higher doses. The mean percentage decrease in amplitude was 42% and 56.98% at doses of 7.29×10^{-4}M ($p<0.1$) and 14.5×10^{-4}M ($p<0.01$) respectively. (Table I),(Fig.**1**).BK VII showed decrease in amplitude of contraction at much higher doses. The mean percentage decrease in amplitude was 13.33% ($p>0.05$), 22.98% ($p>0.05$), 20.66% ($p<0.05$) and 84.93% ($p<0.05$) at doses of $6.6x10^{-5}$M, $13.2x10^{-5}$M, $26.3x10^{-5}$M and $52.6x10^{-5}$M respectively (Table II),"(Fig.2)".

Effect on Coronary Flow

Nifedipine led to a significant increase in the coronary flow at lower doses. The mean percentage increase was 9.40% ($p<0.001$), 13.00% ($p<0.001$) with doses of $0.05x10^{-4}$M and $0.11x10^{-4}$M respectively. A mean percentage decrease of 4.19% ($p<0.001$) and 13.09% ($p<0.001$) was observed at higher doses of $0.23x10^{-4}$ M and $0.46x10^{-4}$M respectively (Table III) "(Fig.**3**)".

With compound BK-VI and BK-VII, no significant increase or decrease on coronary flow was observed except at higher dose at which a significant reduction in coronary flow was noted. The mean percentage decrease with BK-VI, was 39.95% ($p<0.01$) at dose of 14.5×10^{-4}M. (Table I),(Fig.**1**). The mean percentage decrease with BK-VII was 52.79% ($p<0.001$) at dose of $52.6x10^{-5}$M (Table II)," (Fig.2)".

VI. Discussion And Conclusion

1, 4 - dihydropyrimidine - 5 carboxylate compounds can act as alternatives [13] for nifedipine and other dihydropyridine drugs [5], currently being used in the treatment of various cardiovascular diseases. In the present study, the pharmacological actions of a newly synthesised dihydropyrimidine derivative 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1, 4-dihydropyrimidine (BK-VI) and 5- acyl - 6- methyl -4 (2,3- methylenedioxy) phenyl 2-s-benzyl-1,4- dihydropyrimidine (BK VII), were studied on cardiovascular system. 'In vitro' preparation of isolated perfused rabbit heart was used for serving that purpose.Six experiments were conducted with different concentrations of BK-VI, BK-VII and nifedipine in each parameter.

In the heart, calcium channel blockers can reduce or block impulse generation in the SA node and conduction in the AV node. Although nifedipine reduces the slow inward Ca^{2+} current in a dose dependent manner but it does not affect the rate of recovery of the slow Ca^{2+} channel [14]. For testing the effect on cardiac muscle and the coronaries, isolated perfused rabbit heart was used. The effect of the test compounds BK-VI and BK-VII was compared with that of prototype dihydropyridine compound, nifedipine. BK-VI and BK-VII were found to be less potent pertaining to depressant action on amplitude of contraction of the heart and on the heart rate and these depressant effects appeared comparatively at a higher dose range with test compounds BK-VI and BK-VII.

It has been shown that though dihydropyrimidines mimic the biological activity of dihydropyridines, when compared directly with similarly substituted 2-heteroalkyl-dihydropyridines and were found to be 30-fold less active [15]. These observations are in agreement with our results, where our compounds BK-VI and BK-VII, which are also dihydropyrimidine derivatives, show myocardial depressant effect similar to nifedipine, a dihydropyridine and they are less potent myocardial depressants as compared to nifedipine.

Calcium channel blockers show vasodilation on the arterial resistance vessels and coronary vessels [16]. With test compound BK-VI and compound BK VII there was significant reduction in the coronary flow at high dose of 14.5×10^{-4}M and 5.26x10-4M respectively. Nifedipine, on the other hand caused a significant increase in the coronary flow although this effect was lost at higher doses ($0.23x10^{-4}$M and $0.46x10^{-4}$M) and instead a decrease in coronary flow was observed. Thus, it can be concluded that BK-VI and BK-VII have negative inotropic and negative chronotropic effect on mammalian heart and there is decrease in the coronary flow which is significant at high doses. In addition,BK-VI shows increase in coronary flow at low doses which can be beneficial in congestive heart failure.Certain drugs show more pronounced effect in disease and in pathophysiological model than in physiological condition [17], there is therefore a need for appropriate pathophysiological models, the results of which, however, may be affected by difference in species.

FIGURES AND TABLES

Table I : Mean %Age Change In Heart Rate(Beats/Minute), Amplitude(Mm) And Coronary Flow(Ml/Min) With Increasing Dose Of Bk-Vi On Isolated Perfused Rabbit Heart (N=6)

Dose (µg/ml)	Heart rate(Beats/min.)	Amplitude(mm)	Coronary Flow(ml/min.)
50	1.69 ↓ p>0.05	20.42 ↓ p>0.05	2.98 ↑ p>0.05
100	6.29 ↓ p>0.05	7.23 ↓ p>0.05	13.83 ↑ p>0.05
200	13.20 ↓ p>0.05	42.00 ↓ p<0.01	12.46 ↓ p>0.05
400	20.22 ↓ p>0.05	56.98↓ p<0.01	39.95 ↓ p<0.01

Table II : Mean %age change in heart rate(beats/minute), amplitude(mm) and coronary flow(ml/min) with ncreasing dose of bk-vii on isolated perfused rabbit heart (n=6)

Dose (µg/ml)	Heart rate(Beats/min.)	Amplitude(mm)	Coronary Flow(ml/min.)
25	0.07 ↓ p>0.05	13.33 ↓ p>0.05	2.37 ↓ p>0.05
50	4.74 ↑ p>0.05	22.98 ↓ p>0.05	1.53 ↓ p>0.05
100	13.79 ↓ p<0.05	20.66 ↓ p>0.05	22.34 ↓ p>0.05
200	43.55 ↓ p<0.05	84.93↓ p<0.001	52.79 ↓ p<0.001

Table III Mean %age change in heart rate(beats/minute), amplitude(mm) and coronary flow(ml/min) with increasing dose of nifedipine on isolated perfused rabbit heart (n=6)

Dose (μg/ml)	Heart rate(Beats/min.)	Amplitude(mm)	Coronary Flow(ml/min.)
2	4.31 ↓ p<0.01	17.97 ↓ p<0.001	9.40 ↑ p<0.001
4	20.75 ↓ p<0.001	57.39 ↓ p<0.001	13.00 ↑ p<0.001
8	25.17 ↓ p<0.001	91.08 ↓ p<0.001	4.19 ↓ p<0.001
16	100 ↓ p<0.001	100 ↓ p<0.001	13.09 ↓ p<0.001

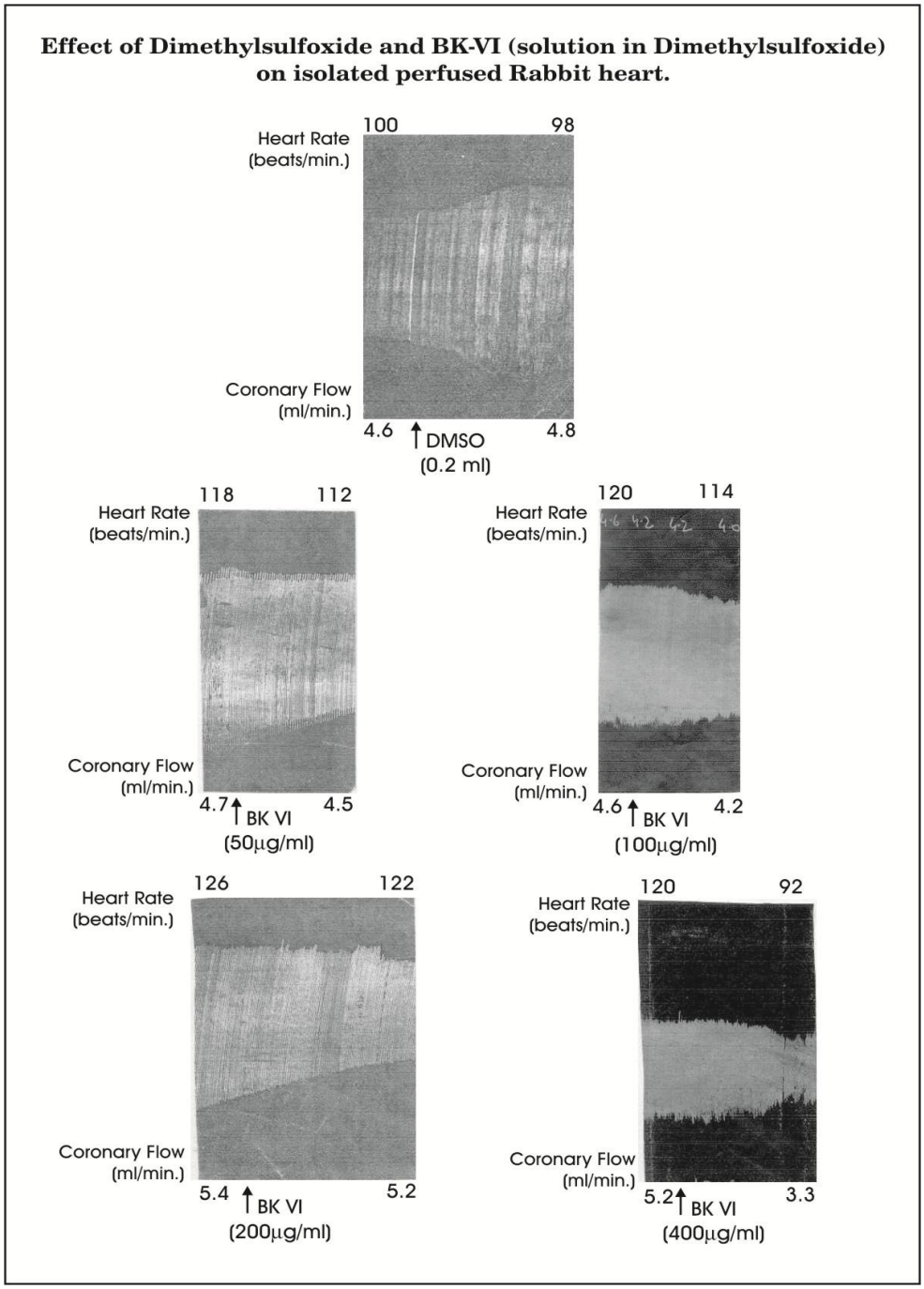

Figure 1

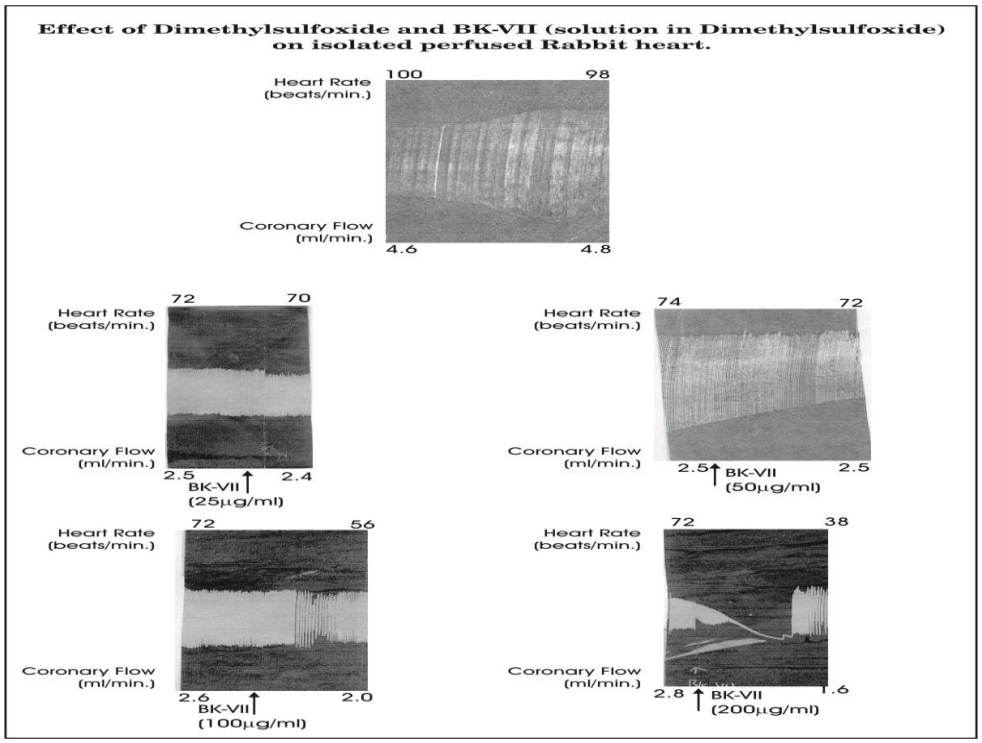

Figure 2

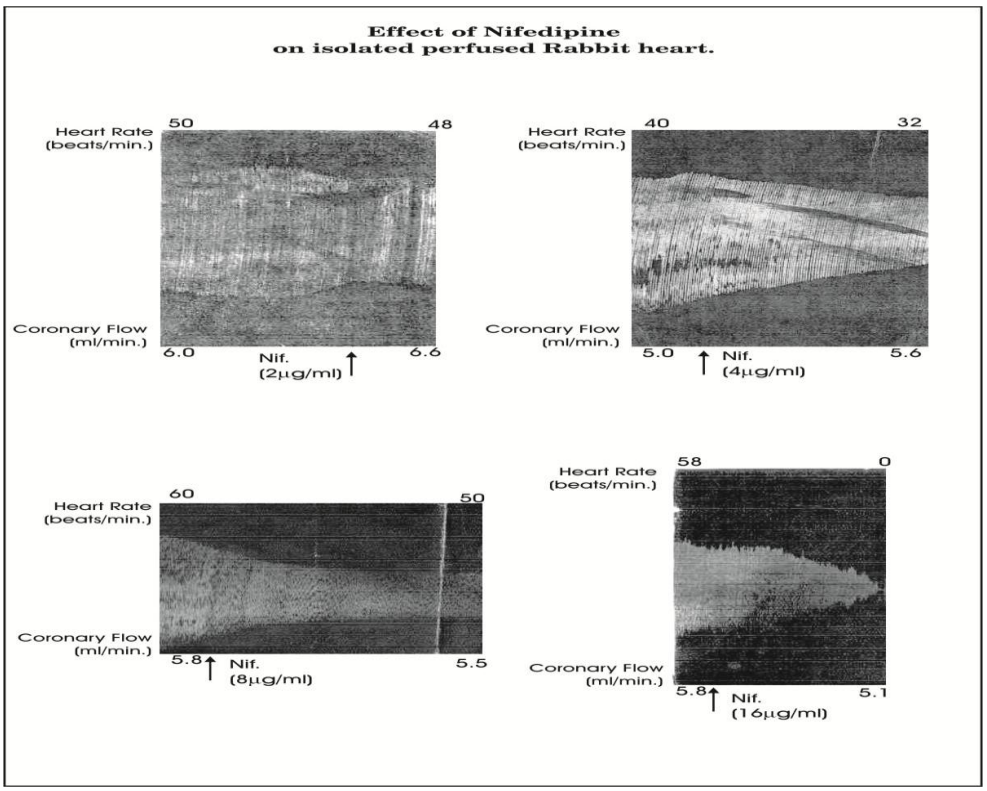

Figure 3

References

[1]. Schereiber Stuart L. Target-oriented and diversity-oriented organic synthesis in drug discovery. Science, 287(5460),2000,1964-1969.

[2]. Dallinger Dorris and Kappe.C.Oliver.Creating chemical diversity space by scaffold decoration of dihydropyrimidines, Pure Appl. Chem. 77, 2005, 155-161.

[3]. Biginelli P. Gazz.Chim.Ital.,23, 1893,360.

[4]. Kumar B.R Prashantha, Masih Pankaj , Karthikeyan E., Bansal Ankur , Vijayan Suja,Pottekad.Synthesis of novel Hantzsch dihydropyridines and Bignelli dihydropyrimidines of biological interest:a 3D-QSAR study on their cytotoxicity. Medicinal Chemistry Research,19, 2010,344-363.
[5]. Bossert F, Meyer H, Wehinger E. 4-aryldihydropyridines,a new class of highly active calcium antagonists. Angen Chem Int Ed Engl, 20,1981, 762-769.
[6]. Baldwin JJ, Claremon DA, Mcclure DE. 2,6-methano-2H-1,3,5-benzothiadiazocine-11-carboxylates useful as calcium channel blockers.US patent 4,609,494(1986) Chem Abstr, 106,1987,18636d.
[7]. Kappe CO.100 years of the bignelli dihydropyrimidine synthesis. Tetrahydron,49, 1993, 6937-6963.
[8]. Rovnyak GC, Kimball D, Beyer B et al.Calcium entry blockers and activators:Conformational and structural determinants of dihydropyrimidine calcium channel modulators. J Med Chem ,38(1),1995,119-129.
[9]. Perry WLM. Pharmacological experiments on isolated preparations.Edinburgh(2)(E & S. Livingstone,1970)116.
[10]. Pathak P. Synthesis of 1,4-dihydropyrimidines and their pharmacological role
[11]. for congestive heart failure,J.Chem.Pharma.Res6(6),2014,838-842.
[12]. Kaur J. Synthesis of biologically active compounds and use of microwave radiations and reactions on solid surface in synthesis.Doctoral diss., Deptt. Of Chemistry, Punjabi University, Patiala,India,1999.
[13]. Ghosh MN,Fundamentals of experimental pharmacology(2nd ed.Calcutta:Scientific book agency,2005) 3-4,10,22-23,90,92-93,130-131.
[14]. Atwal KS, Rovnyak GC, Kimball SD et al. Dihydropyrimidine calcium channel blockers: 2,3-substituted -4-aryl-1,4-dihydro-6-methyl-5-pyrimidine carboxylic acid esters as potent mimics of dihydropyridines. J Med Chem, 33,1990a, 2629-2635.
[15]. Kohlhardt M and Fleckenstein A.Inhibition of slow inward current of nifedipine in mammalian ventricular myocardium. Naunyn-Schemiedeberg's Arch Pharmacol, 298, 1977,267-272.
[16]. Atwal KS, Rovnyak GC, Schwartz J. Dihydropyrimidine calcium channel blockers: 2-heterosubstituted-4-aryl-1,4-dihydro-6-methyl-5-pyrimidine carboxylic acid esters as potent mimics of dihydropyridines. J Med Chem, 33, 1990b, 1510-1515.
[17]. Kerins DM, Robertson RM and Robertson D. (2001) Goodman and Gilman's, "The pharmacological basis of therapeutics", The McGraw-Hill Companies, Inc, USA, 10,853-860.
[18]. Godgraind T, Miller RC and Wibo M. Calcium antagonism and calcium entry blockade. Pharmacol Rev, 38, 1986, 321-416.