RESEARCH ARTICLE

SMOOTH MUSCLE RELAXANT ACTIVITY OF A DIHYDROPYRIMIDINE DERIVATIVE 5-ACYL-6-METHYL-4-PHENYL-2-S-ETHYL-1, 4-DIHYDROPYRIMIDINE (BK- VI) ON ISOLATED RAT UTERUS AND RABBIT AORTIC STRIP

***Salwan Poonam[1],** Salwan Shalini [2], Walia R[3], Bajaj V.K [4], Kaur B [5]

[1]Assistant Professor, Department of pharmacology, Manav Rachna Dental College, Faridabad, Haryana
[2]Associate Professor, Department of pharmacology, PIMS, Garha Road, Jalandhar
[3]HOD, Department of pharmacology, MMIMSR, Mullana, Ambala
[4]Professor, Department of pharmacology, GMC, Patiala
[5]Department of chemistry, Punjabi University, Patiala
**Corresponding Author E-mail – salwanpoonam@yahoo.in*

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ABSTRACT
To investigate the smooth muscle relaxant activity of a newly synthesized dihydropyrimidine derivative 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine (BK- VI) and nifedipine on isolated rat uterus and rabbit aortic strip. Effect of the test compound BK-VI on the smooth muscles of isolated rat uterus and isolated rabbit aortic strip was observed and compared with that of nifedipine. Observations were made with increasing bath concentrations of BK-VI and nifedipine. Six preparations were used for each dose of BK-VI and nifedipine. Mean effect of increasing doses of BK-VI and nifedipine on the height of calcium-induced contraction of depolarized isolated rabbit aortic strip and on K^+-induced contraction of isolated rat uterus were noted and IC-50 calculated. Test compound BK-VI had a significant dose-dependent relaxant effect on K^+-induced contractions of isolated rat uterus. Significant relaxation was seen at bath concentration starting from $9.34x10^{-4}$M (IC-50=$12.2x10^{-4}$M).Nifedipine showed significant relaxation at all bath concentrations starting from $2.8X10^{-7}$M(IC_{50}=$7.5X10^{-7}$M). Compound BK-VI produced potentiation of Ca^{2+}-induced contractions of K+-depolarized rabbit's aortic strip at bath concentration of 0.7×10^{-5}M while significant inhibition was observed at higher bath concentrations. Nifedipine showed dose-dependent significant inhibition at all bath concentrations. BK-VI has a calcium channel blocking activity like nifedipine and it can inhibit the Ca^{2+} dependent contractions of smooth muscles of uterus and aorta.
Key Word: Calcium channel blockers, Dihydropyrimidines, Voltage dependent calcium channels.

INTRODUCTION

Drug development is a long and expensive process.[1]Rising cost; increased attrition rates and non recovery of investments are major hindrances to investment in higher risk drugs or in therapies for uncommon diseases. Much interest has been shown in applying better designs to expedite the approval of new medicinal products.[2] This study aims to develop new synthetic compound having biological activity like nifedipine.The newly prepared 2-Hetero substituted -4- aryl -1, 4-dihydro-6-methyl-5-pyrimidine carboxylic acid ester compounds have been shown to be potent mimics of dihydropyridine calcium channel blockers with the help of radioligand binding techniques and biological assays using potassium-depolarized rabbit aorta.[3]The "Biginelli compounds" as they are called, have been found to have important therapeutic and pharmacological properties[4] as channel blockers, antihypertensive agents, α1a antagonists and neuropeptide Y(NPY) antagonists.[5] Besides having been found to be potent inhibitors of depolarization-induced contractions of isolated smooth muscle preparations, some of the dihydropyrimidines are also reported to have shown anti-ischaemic properties in animal models.[6] Several marine alkaloids, with interesting biological activities as well, include the dihydropyrimidine motif in their structures.[7] Several bicyclic dihydropyrimidines have been synthesized and evaluated for their calcium antagonistic activities by comparison with usual 1, 4-dihydropyridine calcium antagonistic reference compound nifedipine. These compounds can adopt the most important structural features of the 1, 4-dihydropyridine and 1, 4-dihydropyrimidine calcium channel blockers.[8] Bicyclic pyrido (2, 3-d) pyrimidine derivative have been synthesized in one step through the Hantzsh synthesis using 6-aminouracils as enamine nucleophiles and 2 arylmethylene acetoacetates in an appropriate solvent.One such compound BK-VI was evaluated for its calcium-channel blocking activity.

MATERIAL AND METHODS

Test compound BK-VI

Test compound 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine (BK- VI)),(Molecular weight-274) was obtained from department of chemistry,Punjabi university,Patiala.A mixture of benzaldehyde (0.01 mole, 1.06 gm), thiourea (0.01 mole, 0.76 gm), acetylacetone (0.015 mole, 1.5 ml) and concentrated HCl (3-4 drops) in absolute alcohol (10ml) was irradiated at 30% microwave power level. The tetrahydropyrimidine obtained was separated, dissolved in NaOH solution and to this mixture,diethyl sulfate was added. The solid product separated was confirmed by taking its IR, NMR, UV and mass spectra.[9]Compound BK-VI was found to be soluble in carboxymethylcellulose.

Drugs and chemicals

1% carboxymethyl cellulose was used as a solvent for compound BK-VI 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 and female albino rats (250-350 gm) 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 rat uterus and rabbit aortic strip tissues were used for the present study.

Procedure

Isolated Aortic Strip of Rabbit

It is a known fact that smooth muscles can be stimulated to contract by exposing them to membrane depolarizing high potassium bathing medium. Removing Ca^{2+} ions from the high potassium bathing solution abolishes the mechanical response and can be reinstated by adding Ca^{2+} ions back to the solution.[10]This mechanical response can also be prevented by addition of a Ca^{2+} channel blocking agent to the high potassium bathing solution[11] or aborted by adding such an agent after the contraction has been induced.[12]

The initial studies of calcium channels in vascular smooth muscles by Godfraind and co-workers were done on rat aorta and rabbit mesenteric arteries.[13, 14] Ability of Ca^{2+} channel blockers to prevent such muscular contraction or to abolish it once established was investigated.

In the present study, calcium induced contractions in depolarized arterial smooth muscles of rabbit isolated aortic strip were studied. The animals were killed by a blow on the head and exsanguination, as explained by Godfraind and Kaba (1969). Aortic strips 4 cm long and about 2-3 mm wide were prepared by spiral section. [13,15] For removal of endothelial living, procedure described by Furchgott and Zawadzki (1980) was adopted.[16] Aortic strips were then suspended in a 25ml organ bath containing modified Krebs solution at 37°C, which was continuously oxygenated. A tension load of 3 gram was applied to each preparation for relaxation and kept so for 90 mins, while changing the bath fluid every 10 minutes. Further, incubations were done in Ca^{2+} free Krebs solution containing EDTA for 10 minutes. Next, the preparations were depolarized in Ca^{2+}-free, K+-rich Krebs solution. Calcium chloride was added in doses of 10mM. Response was recorded for 10 minutes.

The preparations were later washed with modified Krebs solution and the whole procedure was repeated with addition of the test compound suspension in 1% carboxy methyl cellulose in the bathing fluid. Six such experiments were conducted with each dose of the test compound and mean value estimated.

Isolated Rat Uterus - IC50

It has been shown that depolarization of rat uterus rendered the smooth muscle cell membrane permeable to extracellular calcium resulting in the contractile response, which is directly proportional to extracellular calcium concentration. Fleckenstein and Grun showed that calcium channel blockers like verapamil, gallopamil etc. suppress excitability and contractility in the rat uterus.[14]

Rat uterus was used to quantify the inhibitory action of the test compound and calculate IC50 in the present study. Priming was done 24 hours prior to every experiment, by administration of Diethyl stilbesterol (DES), 0.1 mg/kg body weight, subcutaneously. Dissection was done and preparation mounted in oxygenated De Jalon solution as per the method described by Ghosh (1984). Temperature of the bath was kept around 30°C. Bath capacity was kept constant at 25ml. Tissues were subjected to a tension of 1 g for half an hour for relaxation after which KCl was added to the bath to get a final concentration of 60 mM. K+ induced contractions were recorded using a frontal writing lever. Magnification was kept at 5-6 times. The contractions were recorded on a static smoked drum for half an hour so as to obtain the maximum response[15]. Fine suspensions of the test compound in 1% carboxymethyl cellulose (CMC) were then added in geometric doses and waiting period of 15 minutes was given for each dose. A cumulative dose response curve was taken. Time matched controls were also recorded for each experiment. Six such experiments were done and IC50 calculated.

Statistics

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

RESULTS

Effect on Isolated Rabbit aortic strip

Six experiments were performed using nifedipine and six using compound BK VI at different doses showing their relaxant effects on calcium

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induced contraction of depolarized isolated rabbit aortic strip(Figure-1).

Results of each of six experiments are tabulated (Table 1 and 2)

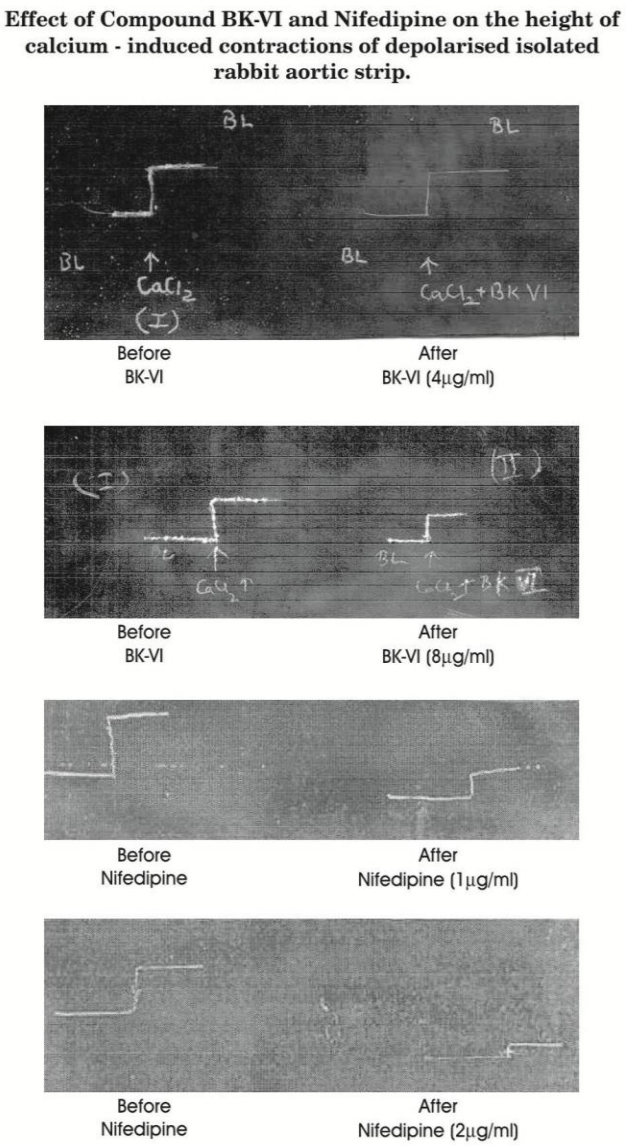

Figure 1: Effect of BK-VI and nifedipine on rabbit aortic strip

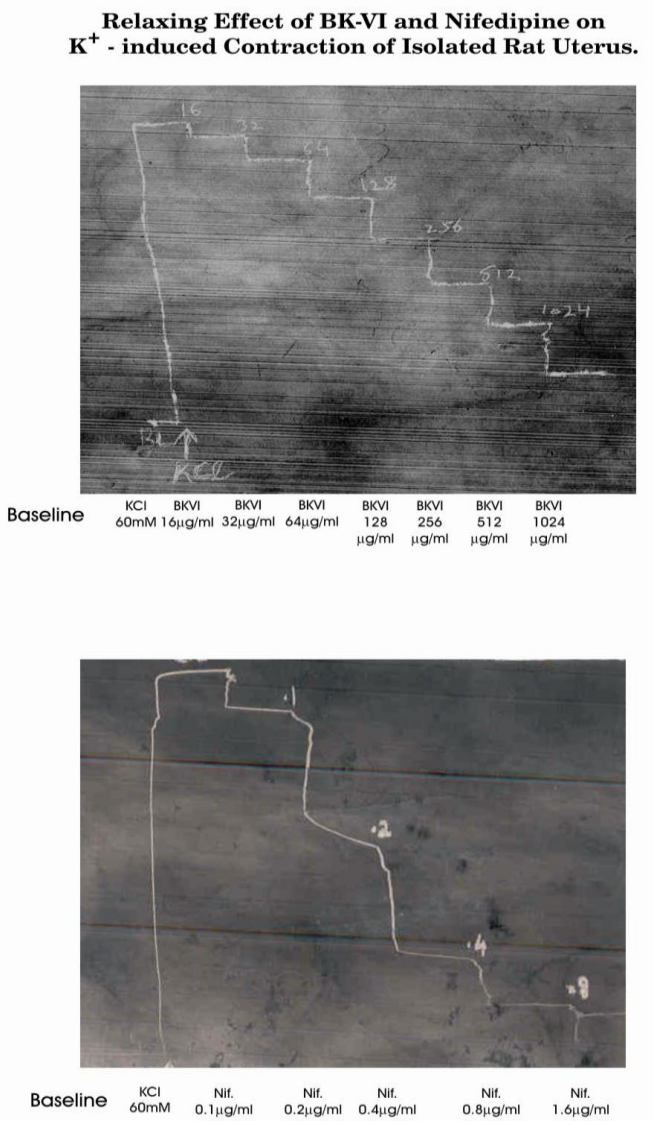

Figure 2: Effect of BK-VI and nifedipine on isolated rat uterus

Table 1: Mean effect of increasing doses of compound BK VI on the height of calcium induced contraction of depolarised isolated rabbit aortic strip (n=6)

Bath Conc. (µg/ml)	Height of calcium induced contraction (mm)		Mean Change	Mean % age change	p
	Before BK VI	After BK VI			
2.5 (0.7×10-5M)	3.67±0.49	4.33±0.42	0.67±0.33	23.61↑	>0.05
4 (1.4×10-5M)	5.33±1.02	3.83±0.91	-1.5±0.34	31.67 ↓	<0.01
8 (2.9×10-5M)	6.67±1.05	4.17±0.70	-2.5±0.43	37.53↓	<0.001
16 (5.8×10-5M)	4.83±0.79	3.33±0.76	-1.5±0.22	35.87↓	<0.01

Effect on Isolated Rat Uterus:

(For calculation of IC-50)

Six experiments were performed using nifedipine and six using compound BK VI at different doses showing their relaxant effects on K+-induced contraction of isolated rat uterus(Figure-2),(Figure-3).

BK-VI(IC-50=$12.2x10^{-4}$ M), Nifedipine(IC-50=$7.5x10^{-7}$M).

Results of each of six experiments are tabulated (Table 3 and 4)

Table 2: Mean effect of increasing doses of Nifedipine on the height of calcium induced contraction of depolarised isolated rabbit aortic strip (n=6)

Bath Conc. (µg/ml)	Height of calcium induced contraction (mm)		Mean Change	Mean % age change	p
	Before Nifedipine	After Nifedipine			
.25 (7.2×10^{-5}M)	5.5±0.96	3.33±0.61	2.16±0.40	39.72↓	<0.001
.5 (1.44×10^{-6}M)	5.5±0.96	0.30±0.68	2.67±0.71	47.69↓	<0.001
1 (2.88×10^{-6}M)	6.8±0.79	2.17±0.47	4.67±0.49	69.25↓	<0.001
2 (5.77×10^{-6}M)	7.5±0.61	0.50±0.22	7.0±0.77	92.35↓	<0.001

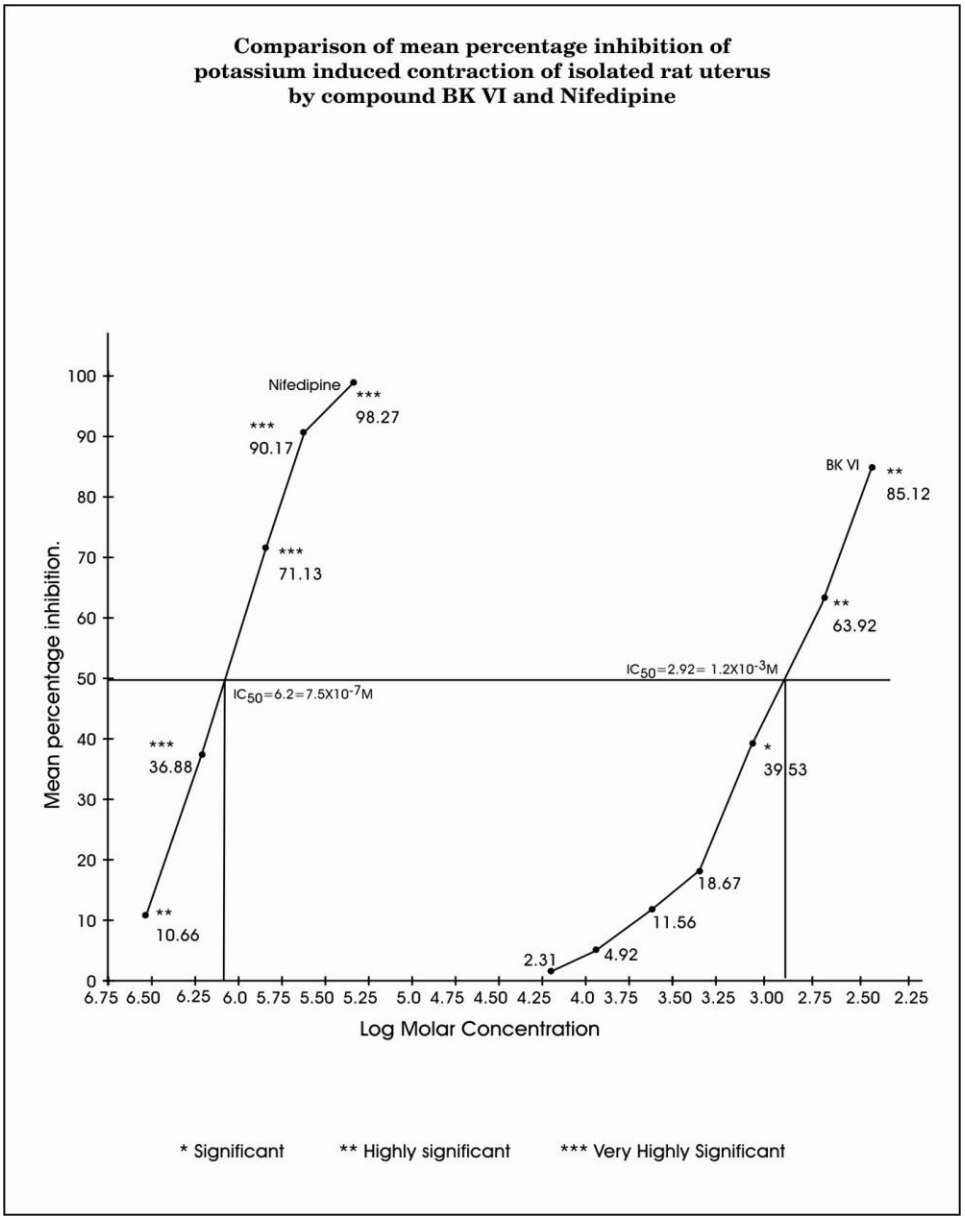

Figure 3: Graph showing IC-50 of nifedipine and BK-VI

Table 3: Mean relaxing effect (Mean±SE) of increasing doses of compound BK-VII on K+-induced contraction of isolated rat uterus (n=6)

Bath Conc. (µg/ml)	Height of K+-induced contraction (mm)		Mean Change	Mean% age change	p
	Before BK VI	After BK VI			
16 (5.8×10^{-5}M)	63.6±15.1	46.0±13.7	0.92±0.45	2.31	>0.05
32 (11.6×10^{-5}M)	63.6±15.1	44.5±13.3	2.0±1.06	4.92	>0.05
64 (23.3×10^{-5}M)	63.6±15.1	42.0±12.7	5.17±2.93	11.56	>0.05
128 (46.7×10^{-5}M)	63.6±15.1	36.8±11.9	8.83±4.07	18.67	>0.05
256 (93.4×10^{-5}M)	63.6±15.1	29.8±11.7	20.25±8.63	39.53	<0.05
512(186.8×10^{-5}M)	63.6±15.1	19.5±0.85	31.9±8.83	63.92	<0.01
1024(373.7×10^{-5}M)	63.6±15.1	0.75±0.47	49.8±10.94	85.12	<0.01

Table 4: Mean relaxing effect (Mean±SE) of increasing doses of Nifedipine on K+-induced contraction of isolated rat uterus (n=6)

Bath Conc. (µg/ml)	Height of K+-induced contraction (mm)		Mean Change	Mean % age change	p
	Before Nifedipine	After Nifedipine			
0.1 (2.8×10-7M)	31.7±9.96	27.8±8.58	3.83±1.49	10.66	<0.01
0.2 (5.7×10-7M)	31.7±9.96	18.83±5.05	12.83±4.95	36.88	<0.001
0.4 (11.5×10-7M)	31.7±9.96	7.66±1.58	24.0±8.91	71.13	<0.001
0.8 (23.1×10-7M)	31.7±9.96	2.16±1.07	29.5±10.21	90.17	<0.001
1.6 (46.2×10-7M)	31.7±9.96	0.5±0.34	30.66±10.10	98.2	<0.001

DISCUSSION

In recent years, 1, 4 - dihydropyrimidine - 5 carboxylate compounds have been presented as valuable substitutes[3] for the well-known nifedipine and other dihydropyridine drugs[17], clinically used in the treatment of cardiovascular disease.

In the present study, the pharmacological actions of a newly synthesized dihydropyrimidine derivative 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1, 4-dihydropyrimidine (BK-VI) were studied on smooth muscles. 'In vitro' preparation was used for serving that purpose viz: Isolated rat uterus and isolated rabbit aortic strip. Six experiments were conducted with different concentrations of BK-VI and nifedipine in each parameter.

It has been shown that the inhibitory effect of drugs like lidoflazine, cinnarizine and chlorpromazine on the contractions of several arteries evoked by KCl-rich solutions could be reversed by increasing the concentration of calcium in the perfusate. Also depolarization in K+ containing solution does not seem to release intracellular Ca^{2+} unless calcium is present in the bathing medium. This reinforces the idea that K+-induced contractions are dependent on entry of extracellular calcium.[14] Thus the drugs which inhibit such contractions may possibly do so by blocking the calcium channels present on the smooth muscles of the test preparations.

Compound BK-VI was found to be having a dose-dependent relaxant effect on the K+-induced contractions of isolated rat uterus. Significant relaxation was seen at bath concentration starting from $9.34x10^{-4}$ M (IC-50=$12.2x10^{-4}$ M). Nifedipine in comparison shows highly significant to very highly significant dose-dependent relaxant effect on the K+-induced contractions of isolated rat uterus, at all bath concentrations starting from $2.8x10^{-7}$M (IC-50=$7.5x10^{-7}$M).

Compound BK-VI produced potentiation of Ca^{2+} induced contractions of K+-depolarized rabbit's aortic strip at bath concentration of $0.7x10^{-5}$M. But at higher bath concentration of 1.4×10^{-5}M, 2.9×10^{-5}M and 5.8×10^{-5}M, significant inhibition of Ca^{2+} induced contractions is seen. These results are in conformity with the studies done by Godfraind and co-researchers[13].

From above cited experiments, it can be safely concluded that the compound BK-VI does have a calcium channel blocking activity and it can inhibit the Ca2+ dependent contractions of the smooth muscles of uterus and aorta.

CONCLUSION

Effect of compound BK-VI was compared with that of nifedipine on uterine smooth muscle. It was found that BK-VI has a calcium-channel blocking activity and a significant dose dependent relaxant effect on uterine smooth muscles was observed at doses higher than those of nifedipine.

BK VI has significant relaxant effect on aortic smooth muscles. This effect was seen at doses higher than those of nifedipine. In comparison to nifedipine, dose-dependent increase in relaxant effect was not observed. Thus it can be concluded that compound 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4dihydropyrimidine (BK- VI) produced calcium channel blocking activity on smooth muscles. In order to ascertain the status of this compound as a drug, further studies are needed not only in other animals and tissue models but also in various pathophysiological models, since some drugs show more pronounced effect in disease and in pathophysiological models than in physiological conditions[14] e.g. in contrast to verapamil, flunarizine has no observable effect on the slow calcium channels of myocardial tissue, but like verapamil, it is a very powerful protecting agent against myocardial damage evoked in vivo by large doses of isoproterenol, which is attributed to intracellular calcium overload. There is therefore a need for appropriate pathophysiological models, the predictive value of which, however, may be affected by species differences.

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