THE ROLE OF K^+ CHANNEL SUBTYPES IN CATECHIN INDUCED VASORELAXATION IN RAT'S AORTIC RINGS

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Abstract;

The current study aimed to investigate the role of K^+ channels subtypes in relaxant effects of catechin on rat's aortic rings. The isometric tension of the thoracic aorta was measured using ADI PowerLab Data Acquisition System. Catechin at concentrations (10^{-5} to $5x10^{-1}$M) produced more potent relaxant effects in phenylephrine (10^{-6} M) precontracted aortic smooth muscle with Log IC_{50}'s of -1.159 mg/ml as compared to potassium chloride (60 mM) with Log IC_{50}'s of -6.373 mg/ml . The catechin induced relaxation in aortic rings precontracted with phenylephrine was 17.464 ± 0.068 %, where as for aortic rings precontracted with potassium was only 5.574 ± 0.131%. In aortic rings preincubated with glibenclamide (10^{-5}M) and tetraethylammonium (1mM), catechin relaxant effect was enhanced with Log IC_{50}s of -3.119 and -3.001 mg/ml, respectively. On the other hand, in aortic rings precontracted with PE and preincubated with $BaCl_2$ and 4-AP, catechin induced statistically non-significant relaxant effects as compared with aortic rings of the control aortic ring which was precontracted with PE. From the results of the current study, it can be concluded that catechin produced more potent vasorelaxant effect on aortic rings preincubated with phenylephrine that KCl. This vasorelaxant effect of catechin is produced via the activation of both K_{ATP} and Kca, but not Kir and Kv channels subtypes.

Keywords: *K^+ channel blockers, Catechin, vasorelaxation, aorta.*

Introduction

Catechins, which are also known as flavan-3-ols, are simple forms of flavonoid; based on their structure, they are classified as flavanols which include catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate.

Epicatechin concentrations are high in apples, blackberries, broad beans, cherries, black grapes, pears, raspberries, and chocolate. The remaining catechins such as epigallocatechin, epicatechin gallate, and epigallocatechin gallate are found in high concentrations in both black and green tea (Williamson and Manach,2005 and Ghayur *et al*., 2007).

Catechins has an important protective role against many diseases such as heart disease, stroke, atherosclerosis, hypertension, kidney disorders, obesity, diabetes (Crespy and Williamson, 2004) and act as antithrombic agent (Yang, et al, 19990). Furthermore, the antioxidant effects of Catechins has been recognized as they prevent the oxidation of antioxidants such as vitamin E and even some times elevate its level (Alessio, *et al*, 2003 and Tijburg, *et al*, 1997). *In vitro* studies on Catechins have shown that it inhibit catechol O-methyltransferase (COMT), the enzyme that degrades norepinephrine; and thus, reflecting the important role of the sympathetic nervous system and its neurotransmitter norepinephrine in the control of thermogenesis and fat oxidation (Dulloo *et al.,* 1999).

Catechin can induce vasorelaxant responses via the stimulation of endothelial Nitrous oxide (NO) and nitrous oxide synthase (NOS) (Huang *et al*., 1999 and Lorenz *et al*., 2004) or increased the production of PGI_2 (Mizugaki *et al*., 2000). Catechin-evoked vasorelaxant effects appear to be both endothelium-dependent and independent (Huang *et al*., 1998). It has been indicated that the effect of Epigallcatechin-3-gallate on rat's aorta may be used as an interesting model for the subsequent development of new PDE- inhibitory drugs for improving the pharmacological treatment of diseases such as cardiovascular pathology (Alvarez, et al, 2006).

Taking into the consideration the above informations on Catechin effects, and since no attempt have been made to study the role of K^+ channels subtypes in vasorelaxation effect of Catechins, the current work was undertake to shed light on the role of above channel subtypes in vasorelaxation induced by catechin in rat's aortic rings.

Materials and Methods

Materials

Albino Rats

Adult male albino rats, *Rattus rattus norvegicus* (250-350g) used in the current study were bred in the Department of Biology, Faculty of Science, University of Zakho. The rats were reared in standard PVC rat cages, maintained in the laboratory under controlled temperature (22 ± 2 ℃), and photoperiod of 12-hours light/dark cycle using automated light-switching device. All rats were provided with standard food pellets prepared as described by Shekha, (2010) with a free access to water *ad libitum*. The animals were acclimatized to the laboratory conditions for 1-2 weeks before using them in the experiments.

Methods

Isolated Aorta Preparation

The animals were injected intraperitoneally with heparin (1500 units/ kg body weight) and left for 30 min, to avoid blood clotting and possible damage of endothelial layer of the aorta (Fulton *et al*., 1996). Animals were then anesthetized with Ketamine (40 mg /kg) and Xylazine (10 mg/Kg) intraperitoneally.

The chest cavity was opened and after the removal of excess tissue and fat, the aorta was isolated and transferred to a beaker containing Krebs solution aerated with carbogen [95 % oxygen (O_2) and 5 % Carbone dioxide (CO_2)]. The beaker was placed in the water bath at 37 ^oC and the aorta cut into small rings of about 2-4 mm long.

Measurement of Isometric Contraction in Isolated Rat Aorta

The procedure of Shekha and Al-Habib, (2012) was followed with some modifications to study the vascular reactivity in the isolated aorta. Two stainless steel wires were carefully passed through the lumen of the aortic rings. One of them was anchored to the base of glass organ chamber and the other was connected to a force transducer (Model MLT0201/RAD) coupled to the transbridge amplifier connected to a PowerLab Data Acquisition System and computer running chart software (Version 7) used for isometric tension measurement. Special attention has been taken during the preparation to avoid damaging the endothelium. The extents of contraction and relaxation were expressed by the tension recorded by the system. The relaxation rate was defined as $T_{relaxation}/ T_{contraction}$ expressed as a percentage.

Prior to the experiment, 10 ml of Kreb's solution was placed inside the glass tissue chamber and the organ tissue bath system was maintained at 37 ^oC by circulating water through the water jacket from a circulating water bath set at 37 ^oC. The aorta was continuously aerated by carbogen (95 % O_2 and 5 % CO_2). The initial tension was set at 2 g weight and left for 60 minutes. The aortic segments were initially exposed to 60 mM K^+ to test their functional integrity. After that, the chamber medium was changed several times until a stable resting tone was recorded, then the experiments were started.

Statistical Analysis

The statistical analysis was performed using two-way analysis of variance (ANOVA) supported by Bonferroni test when carrying out a pairwise comparison between the same dose of different groups using Graphpad prism program, version 6.01. Analysis of variance for repeated measurements was applied to data consisting of repeated observations at successive time points. P-values less than 0.05 were considered as statistically significant. In all figures, the symbols (*, ** and ***) representing mean differences are significant at the 0.05, 0.01 and 0.001 levels, respectively. The maximum contractile responses to catechin were calculated as a percentage of the contraction produced by PE or KCl and expressed as the means ± standard error of the mean (SEM). The tension produced by vasoconstrictors (PE and KCl) was defined as 0% tension, and the baseline tension before adding vasoconstrictors was 100% tension**.**

Results

Relaxant Effect of Catechin on Aortic Rings Contracted by PE and KCl

Typical traces from representative experiments on the relaxing effect of different concentrations of catechin on PE and KCl precontracted aortic rings are shown in figure (1). Dose-response curves for the effect of catechin on PE- and KCl-induced contractions are shown in figure (2). Catechin at concentrations from 10^{-2} to $5X10^{-1}$ M resulted in a highly significant relaxant effect ($P<0.001$) in PE (10^{-6} M) as compared with KCl (60 mM) precontracted thoracic aortic rings.

Catechin produced a more potent inhibitory effect on PE than KCl induced contractions,

with Log IC_{50}'s of -1.159mg/mL (Log IC_{50} of CI 95% between -6.708 to 4.389) and -6.373 mg/mL (LogIC_{50} of CI 95% between -22.430 to 9.686), respectively. Catechin produced highly significant relaxant effects on PE-induced contractions which was 17.464 ± 0.068% , whereas catechin produced a mild relaxant effect on the aortic ring precontracted with KCl (5.574 ± 0.131%).

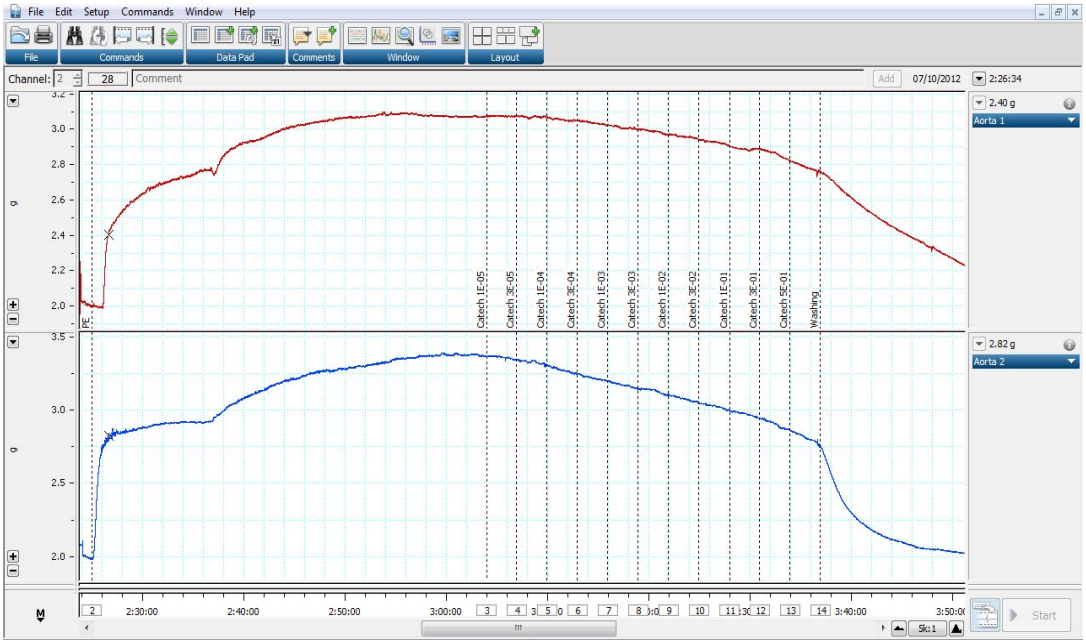

Fig. 1: Typical traces showing the relaxant effects of different concentrations of catechin in rat aortic rings, precontracted with PE (10^{-6}M).

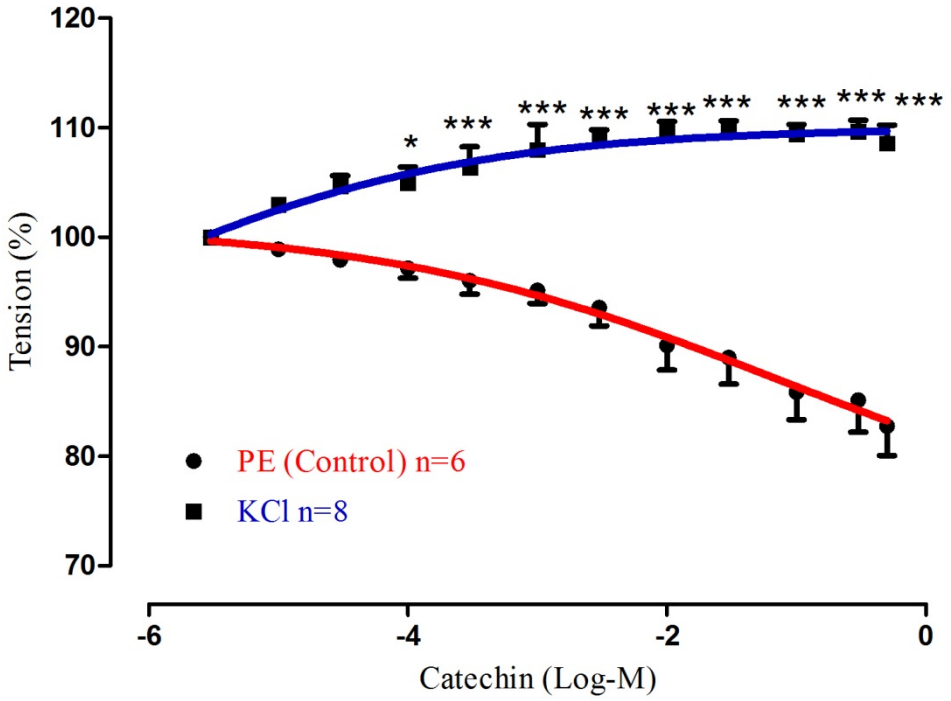

Fig. 2: Cumulative dose-response curve for the effects of catechin on PE (10^{-6}M) and KCl (60mM) precontracted aortic rings.

Role of Potassium Channels Subtypes in vasorelaxation Induced by Catechin

The role of K^+ channels subtypes in the relaxant effect of catechin was investigated by preincubation of aortic rings in GLIB (10^{-5}), TEA (1mM), $BaCl_2$ (1mM) and 4-AP (1mM) and precontracted with PE. The Dose-response curves for Catechin effect on PE precontracted aortic rings preincubated with K^+ channel blockers are shown in figures (3, 4, 5 and 6).

In aortic rings precontracted with PE and preincubated with GLIB in the presence of catechin showed a highly significant ($P<0.001$) relaxant effect at doses ($3X10^{-3}$-$5X10^{-1}$) and with TEA also showed a significant ($P<0.01$) relaxant effect at concentrations (10^{-2}-$5X10^{-1}$) on aortic rings. The percentages of relaxation were 31.089±0.136% and 58.392±0.110 %, and with Log IC50's of -3.119 mg/ml (with a Log IC50 of CI 95% between -3.994 to -2.245) and -3.001mg/ml (with a Log IC50 of CI 95% between -3.875 to -2.128), respectively. On the other hand, aortic rings precontracted with PE and preincubated with $BaCl_2$ and 4-AP showed limited relaxant effects which were statistically non-significant to catechin as compared with aortic rings precontracted with PE as control with Log IC50's of -1.159 mg/ml (with a Log IC50 of CI 95% between -6.708 to 4.389) and the percentages of relaxation were 4.247 ± 0.280 and 17.464±0.068 %.

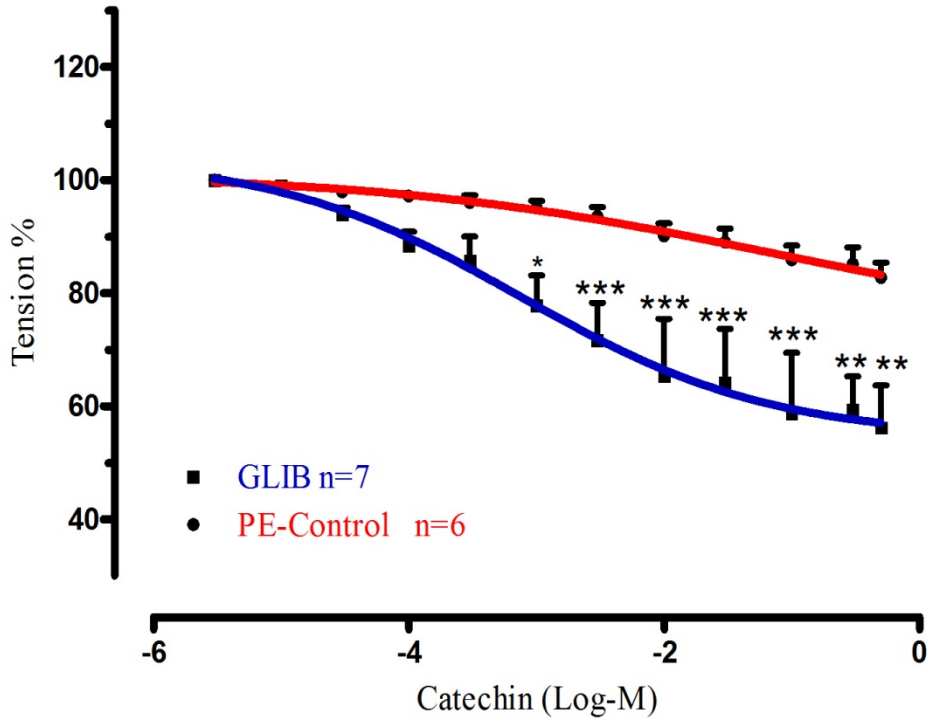

Fig. 3: Cumulative dose-response curve for the relaxant effects of catechin in aortic rings preincubated with GLIB, precontracted with PE.

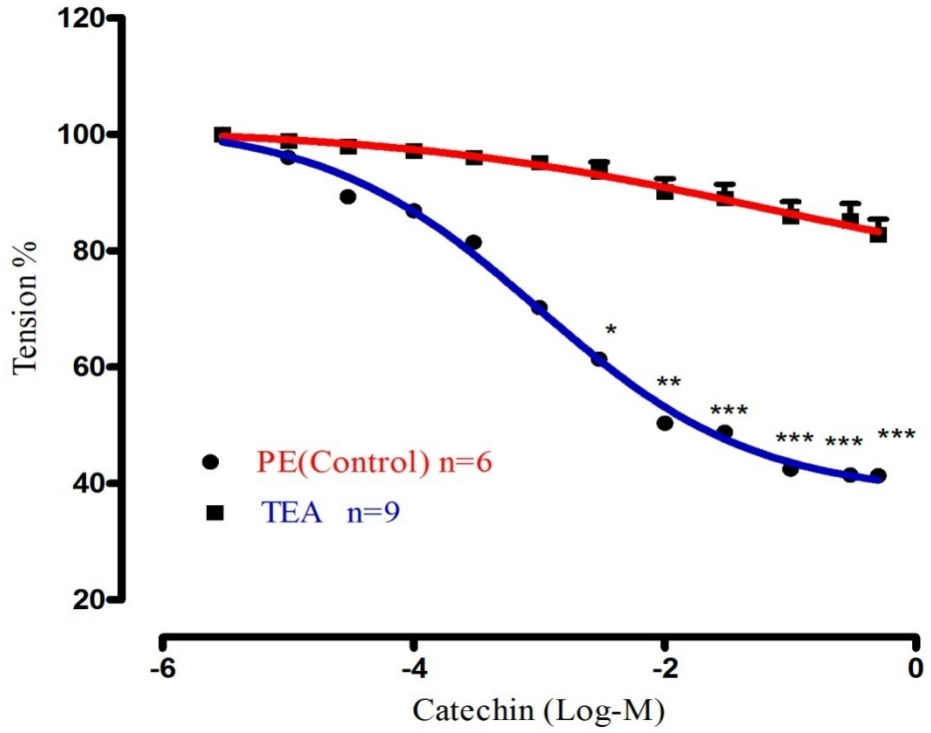

Fig. 4: Cumulative dose-response curve for the relaxant effects of catechin in aortic rings preincubated with TEA, precontracted with PE.

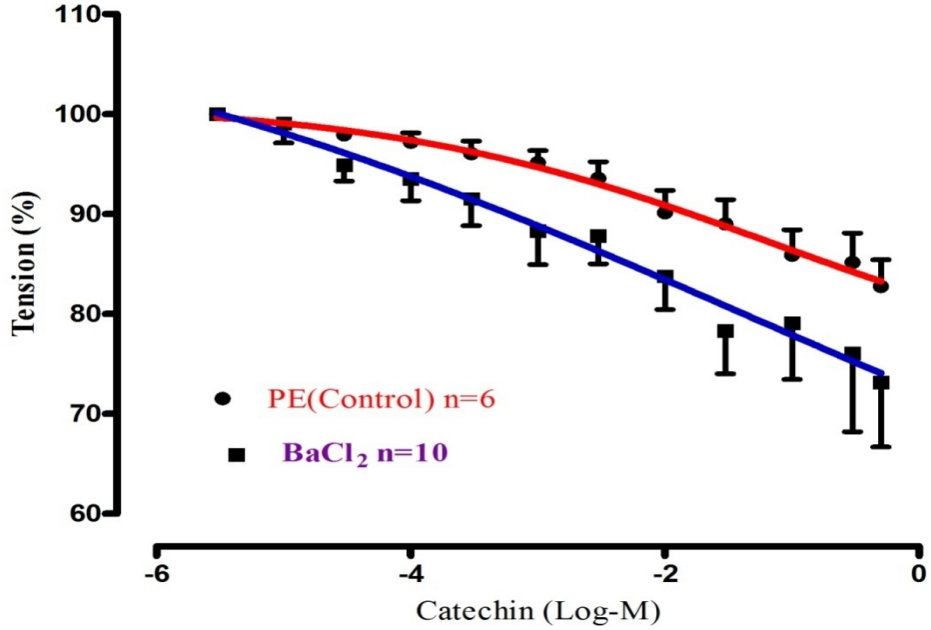

Fig. 5: Cumulative dose-response curve for the relaxant effects of catechin in aortic rings preincubated with $BaCl_2$, precontracted with PE.

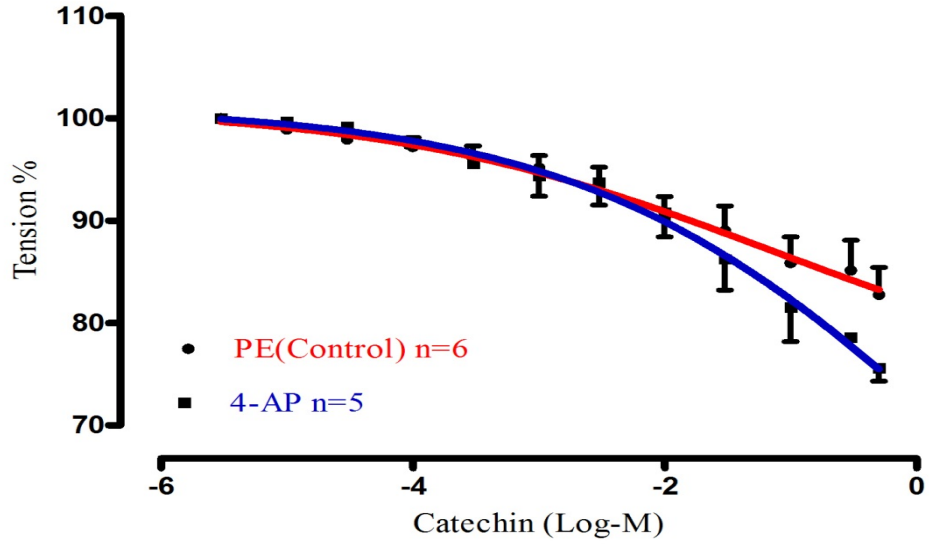

Fig. 6: Cumulative dose-response curve for the relaxant effects of catechin in aortic rings preincubated with 4-AP, precontracted with PE.

Discussion

The results of the study showed that catechin in a dose-dependent mode inhibited the contractions induced by PE and high K^+ concentration in isolated rat thoracic aorta. The flavonoid tested in this study was found to be more sensitive in relaxing the contraction induced by PE. The results of one of the previous study suggested that the reduction of transmembrane Ca^{2+} influx and/or agonist induced release of intracellular Ca^{2+} in these cells may contribute to the vasorelaxant action of catechin, although these effects do not seem to be important for lower concentrations of the catechin (Alvarez, *et al*., 2006).

From the results of the current study, it has been observed that vasorelaxation-induced by catechin was enhanced significantly by TEA and GLIB, but not by 4-AP and $BaCl_2$. These results suggest that vasorelaxant responses to catechin are mediated through increasing K^+ efflux at least, via K_{ATP}, K_{Ca} channels. There is growing evidence that the K_{ATP} channels activity may be modulated by NO. Kubo *et al*., (1993) demonstrated that nitric oxide donors cause activation of K_{ATP} channels in rat aorta by means of a cyclic GMP-dependent mechanism, possibly cyclic GMP-dependent PK.

Álvarez *et al*., (2006) demonstrated that catechin-induced relaxation in rat aortic rings was endothelium-independent and mediated by the inhibition of phosphodiesterase (PDE) 1-5 isoform activity. Moreover, it was reported that catechin has the ability to induce contractile responses in isolated rat aorta (Sanae *et al*., 2002; Shen *et al*., 2003 ; Álvarez-Castro *et al*., 2004).

From the results of the present study it can be concluded that the relaxant effect of catechin on aortic smooth muscle is mediated via the activation of K_{ATP} and Kca, but not Kir and Kv channels.

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پوختە:

ئەم توێژینەوەی ئێستا لێکۆڵینەوەی کاریگەری یەکانی خاوبوونەوەی ، کاتیچین Catechin ی بە ئامانج گرتووە لەسەر ئەڵقەی شاخوێنبەری دەرهێنراو لە جوورج . ئینجا توێژینەوەکە لە ڕۆڵی کەناڵەکانی پۆتاسیۆم وکالسیۆمی کۆڵییەوە لە هاندانیان بۆ خاوبوونەوەی سنگە شاخوێنبەر . بۆ لێکۆڵینەوەی ئەو کاریگەری یانەی کە لە سەرەوە باسکران لە سەر لووسە ماسوولکەی سنگە شاخوێنبەر ، ئەم بۆڕی خوێنە بڕرا و کرایە چەند ئەڵقەیەك و بە گەرماوی ئۆرگانی لووسە ماسوولکەوە بەسترا بۆ پێوانی گرژبوونەوەکانی درێژی چەسپاوی ئەم ماسوولکانە بە بەکارهێنانی PowerLab Data Acquisition System.

کاتیچین بە پەیتی یەکانی (١٠^-٥بۆ٥×١٠^-٥ مۆلار) خاوبوونەوە کاریگەری بەهێزی بە دەرخست لەسەر لووسە ماسوولکەکانی شاخوێنبەر کە پێشتر هاندرا بوون بە فینایلفرین (١٠^-٦مۆلار) بە پەیتی یەکانی لۆگاریتمی IC٥٠ (-١٥٩، ١ ملگم\مل) بە بەراوردکردن بەو لووسە ماسوولکانەی کە پێشتر هاندرا بوون بە کلۆریدی پۆتاسیۆم بە پەیتی (٦٠ملی مۆلار) بە لۆگاریتمی IC٥٠ (-٣،٩١٠ملگم\مل)، هەروەها ڕێژەی خاوبوونەوە کاریگەری فینایلفرین (١٧،٤٦٤±٠،٠٦٨) وە کاریگەری یەکانی کلۆریدی پۆتاسیۆم (٥،٥٧٤ ± ٠،١٣١٪). . لەوەش زیاتر کرکەواندنی پێش وەختەی ئەڵقەکانی شاخوێنبەر بە گلیبینکلامایید (١٠^-٥ مولار) و تێتڕائیتایل ئەمۆنیۆم TEA (١ملی مۆلا ر) بووە هۆی خێراکردنی خاوبوو نەوە کە ڕوویدا بە هۆی کاتیچین بە لۆگاریتمی IC٥٠ (-٣،١١٩ و-١٠٠، ٣ملگم\مل)، لە کاتێکدا زیادبوونی ڕێژەی خاوبوونەوە بینرا بۆ (٣١،٠٨٩ ± ٠،١٣٦٪ و ٥٨،٣٩٢±٠،١١٠٪) یەك لە دوای یەك .

لە لایەکی ترەوە مامەڵەی پێش وەختەی ئەڵقەکانی شاخوێنبەر بە نیفیدیپین بووە هۆی ڕاگرتنی بەر چاو لە خا وبوونەوە وەلأمدانەوە ی ئەم ماسوولکانە بۆ کاتیچین بە لۆگاریتمی IC٥٠ (-١٩٣،٠ ملگم \مل) وە دابەزینی ڕێژەی خاوبوونەوە بۆ (-٣٠،٢٢٦±٠،٠١٧٪) .