

The Inhibitory Effect of Hypericum Triquetrifolium Turra. Extract on Rat Aortic Smooth Muscle Contraction

HYPERICUM TRIQUETRIFOLIUM TURRA. EKSTRESİNİN SİÇAN AORTA DÜZ KASI KONTRAKSİYONU ÜZERİNDEKİ İNHİBİTÖR ETKİSİ *

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Summary

Purpose: The aim of the present study was to investigate the probable vasorelaxant effect of the total extract of Hypericum triquetrifolium Turra. on rat isolated aortic rings.

Place: This study was designed and performed in Ege University, Center for Drug R & D and Pharmacokinetic Applications.

Materials and Methods: In the first part of the experiments, contractions with phenylephrine and KC1 were compared after the tissues were incubated with different concentrations of Hypericum extract. In the second part, the inhibitory effect of Hypericum extract (10⁻⁶-10⁻⁸ g/ml) on the sustained contractions of aorta with phenylephrine and KG was investigated.

Results: The maximal inhibition obtained by Hypericum extract for the phenylephrine contractions was 93.95 ± 5.23%, while the maximal inhibition was found as 85.78 ± 4.87 % for KC1 contractions in the first part of the experiments. In the second part, Hypericum extract inhibited both phenylephrine and KC1 induced contractions in a concentration-dependent manner.

Conclusion: Our results demonstrate that high concentrations of total extract of H. triquetrifolium antagonizes the contractile activity in the rat aortic smooth muscle preparations.

Key Words: Hypericum triquetrifolium, Aorta, Rat, Vasodilation

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Özet

Amaç: Çalışmamızın amacı Hypericum triquetrifolium Turra. ekstresinin olası damar genişletici etkisinin sıçan izole aorta preparatlarında araştırılması idi.

Çalışmanın Yapıldığı Yer: Ege Üniversitesi, İlaç geliştirme ve Farmakokinetik Araştırma Uygulama Merkezi.

Materyal ve Metod: Çalışmanın ilk bölümünde dokular değişik konsantrasyonlarda Hypericum ekstresi ile inkübe edildikten sonra fenilefrin ve KC1 kasılmaları kaydedildi. İkinci bölümde ise Hypericum ekstresinin (10⁻⁶-10⁻⁸ g/ml) fenilefrin ve KC1'nin sabit konsantrasyonları ile oluşan kontraksiyonlar üzerinde oluşturduğu etkiler incelendi.

Bulgular: İlk bölümde Hypericum ekstresi tarafından fenilefrin ile oluşan kasımlarda oluşturulan maksimum inhibisyon %93.95 ± 5.23 bulunurken, bu değer KC1 kasılmalarında oluşan maksimum gevşeme cevapları açısından %85.78 ± 4.87 olarak bulundu. Çalışmanın ikinci bölümünde ise Hypericum ekstresi ile fenilefrin ve KC1 kasılmalarının konsantrasyona bağımlı olarak inhibe olduğu gözlemlendi.

Sonuç: Sonuçlarımız, yüksek dozlarda H. triquetrifolium ekstresinin sıçan aorta düz kas preparatlarında kontraktil aktiviteyi antagonize edebileceğini göstermektedir.

Anahtar Kelimeler: Hypericum triquetrifolium, Aorta, Sıçan, Vazodilatasyon

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Different Hypericum species such as Hypericum triquetrifolium Turra. and Hypericum perforatum L. have been widely used in different parts of Turkey for their antidepressant, sedative, antihelminthic and antiseptic effects. They are also found effective in the treatment of burns (1).

Moreover, it has been shown that *Hypericum triquetrifolium* Turra. exhibits antinociceptive activity in the mouse probably by synaptosomal noradrenaline and serotonin reuptake inhibition (2).

Tricyclic antidepressants have been demonstrated to inhibit noradrenaline and serotonin reuptake (3-6). *Hypericum* species have also been shown to block the uptake of both monoamines in cortical synaptosomes (7-9). In this regard, there are parallels with tricyclic antidepressants which are believed to potentiate the effect of biogenic amines in different systems and that enhancement of noradrenaline and serotonin function may contribute to the antidepressant and analgesic effect of *Hypericum* extract.

Tricyclic antidepressants can produce serious cardiac side effects such as tachycardia and postural hypotension (10). Although *Hypericum* species are reported to be free of any cardiac side effects normally seen with tricyclic antidepressant medications (10-13), there are no *in vitro* or *in vivo* studies particularly investigating this effect. The aim of the present study was to evaluate the probable vasorelaxant effect of the total extract of *Hypericum triquetrifolium* Turra. on rat isolated aortic rings.

Methods

Fresh plants from wild collections, gathered at the start of the flowering period in July 1998 from Urla, İzmir (a city located in the western part of Turkey) were used. Mainly, the aerial parts of the plants that have a high proportion of buds and flowers were selected. The plant was identified by Professor Özcan Seçmen, Ege University, Faculty of Science, Department of Biology, Section of Botany. The voucher specimen of the plants used in the present study was kept for record in the herbarium of Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany (voucher no. 5418).

The crude drug was dried in shade and fine powder of the plant was obtained by a mill (Brabender OHG, Duisburg). A modified method of Wagner and Bladt (14) was used for the extraction of the powdered plant. Methanol at 80°C was used for soxhlation, using 750 ml methanol for 100 g crude drug and the extract was dried *in vacuo* (yield 38.22%). After the lyophilization of the ex-

tract (yield 78.07%), it was dissolved in 4% dimethyl sulphoxide (DMSO) and administered to rats immediately. All procedures concerning animals were carried out in an ethically proper way by following guidelines as set by the World Health Organization. The approval was also obtained from Ege University Animal Ethical Committee.

Male Wistar rats (250-300 g) were killed by cervical dislocation and bled. Aortic rings of approximately 4 mm in length were prepared and mounted in 20 ml organ baths containing Krebs Henseleit (K-H) solution of the following composition (in mM): NaCl 119, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1, NaHCO₃ 25, KH₂PO₄ 1.2, D-glucose 11.1, ascorbic acid 0.2. The bath solution was maintained at 37 ± 1°C and constantly oxygenated with 95% O₂ + 5% CO₂. The preparations were allowed to equilibrate for at least 1.5 hours under 1 g resting tension. Each ring was connected to a force displacement transducer (MAY-COM FDT 10-A, Commat İletişim Ltd. Ankara, Turkey) for the measurement of isometric force which was continuously displayed and recorded on-line on a personal computer (IBM 486 DX2 66) via a data acquisition system (TDA 94, Commat İletişim Ltd. Ankara, Turkey) using a software (Polywin 95 Ver 1.0; Commat İletişim Ltd. Ankara, Turkey) which also had the capacity to analyze the data. In experiments using high KCl solution, the equimolar amount of Na⁺ was replaced by K⁺ to maintain a constant ion strength.

Twenty minutes after setting up the organ baths, tissues were first contracted with a single dose of phenylephrine (10⁻⁶ M) to test for their contractile responses after which they were rinsed three times in K-H solution to restore tension to the precontracted level.

(i) The aortic rings were then contracted with phenylephrine or KCl, applied cumulatively (ranging from 10⁻⁹-10⁻³ M for phenylephrine and from 5-80 mM for KCl) to obtain concentration-response curves. Once the maximal response to phenylephrine or KCl had been reached, preparations were washed with K-H solution every 20 minutes until the tension returned to the basal level. Tissues were then incubated with *Hypericum* extract (10⁻⁵-10⁻³ g/ml) for 30 minutes and another cumulative concentration-response curve for phenylephrine or KCl

was repeated, (ii) In another series of experiments, sustained contractions of aorta to 10^{-6} M phenylephrine, and 80 mM KCl were obtained and Hypericum extract (10^5 - 10^{*3} g/ml) was then applied cumulatively to induce inhibition. The effect of 4% DMSO was also tested for both experimental procedures.

In some experiments, the endothelial layer was removed mechanically by rubbing the lumen of the artery with plastic tubing. Successful removal of the endothelium was verified by the inability of the preparation to relax with 10^{-6} M acetylcholine at the start of each experiment.

Drugs

L-Phenylephrine Hydrochloride (Sigma. St. Louis, MO. USA) was used and dissolved in K-H solution. Hypericum extract was dissolved in 4% DMSO. 4% DMSO in organ baths did not affect muscle contraction induced by agonists.

Statistics

(i) To examine the cumulative concentration-response curves, the PD_2 values ($-\log EC_{50}$, calculated by regression analysis over the range 20-80% of the maximal response) and the maximal responses (E_{max}) were determined from individual concentration-response curves to phenylephrine and KCl. The peak contractile responses to phenylephrine and KCl, before and after incubation with the various concentrations of Hypericum extract were expressed as means \pm S.E.M. of n experiments, and compared by using the Duncan's new multiple range test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations. A p value of 0.05 or less was considered statistically significant.

(ii) To examine the effect of Hypericum extract on the sustained contractions, concentration response curves were used to determine the concentration of Hypericum extract producing 50% inhibition of the maximal contractile response (IC_{50}) obtained by phenylephrine and KCl, using a linear regression analysis over the response range of 20 to 80% of the maximal inhibition. The data are expressed as means \pm S.E.M. of n experiments and statistical analysis was performed with Student's

t test. A probability level of less than 0.05 was considered statistically significant.

Results

The rat aortic rings lacked spontaneous activity. The resting tone was unaffected by 4% DMSO in endothelium-denuded and/or intact rat aortic rings.

(i) Effect of Hypericum extract on the phenylephrine and KCl - induced contractions

Phenylephrine and KCl contracted the rat aorta preparations with a PD_2 of 8.72 ± 0.49 and EC_{50} of 10.25 ± 0.68 mmol / L and a maximal increase in tension of 1.41 ± 0.05 g ($n=19$) and 1.43 ± 0.03 g ($n=14$), respectively. Following construction of control curves for phenylephrine and KCl, the aortic rings were incubated for 30 minutes with different concentrations of Hypericum extract and concentration-response curves for phenylephrine and KCl were again obtained. Addition of Hypericum extract did not affect the basal tension of the aortic muscle preparations. However, it was evident that the extract (10^5 - 10^{*3} g/ml for phenylephrine contractions and 10^{*5} - $1(r^3)$ g/ml for KCl contractions) exerted a significant inhibition, decreasing the magnitude of the maximal contraction and the slopes of the concentration-response curves for phenylephrine and KCl (Figure 1a, 1b). DMSO (4%) did not exert any effect on the contractions obtained by phenylephrine and KCl.

(ii) Effect of Hypericum extract on sustained contractions obtained by phenylephrine and KCl

In another set of experiments, submaximal steady contractions were induced by phenylephrine (10^5 M) and KCl (80 mM) in the rat isolated aortic rings with or without endothelium and cumulative doses of Hypericum extract were added. The extract significantly and dose dependency inhibited both phenylephrine and KCl induced contractions in a concentration-dependent manner with pIC_{50} values of 3.93 ± 0.15 ($n = 19$) and 3.82 ± 0.12 ($n=14$), respectively (Figure 2a, 2b). The differences between IC_{50} values obtained against phenylephrine and KCl were not significant ($p>0.05$). The maximal inhibition obtained for phenylephrine contractions was $93.95 \pm 5.23\%$ while the maximal inhibition was $85.78 \pm 4.87\%$

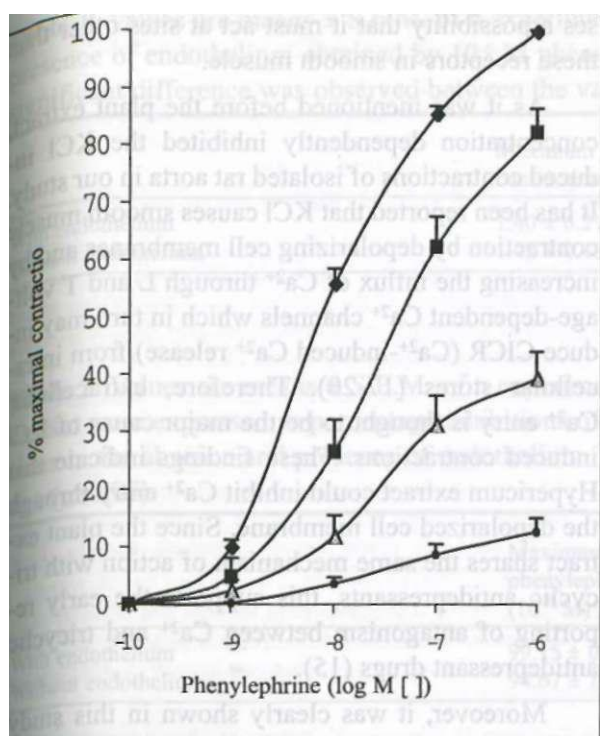


Figure 1a. Logarithmic concentration-response curve of contractile responses of rat aorta for phenylephrine in the absence of endothelium (control, n=41) and presence of Hypericum extract (•, 105 g/gi, n=12; ▲, 3 x 10⁻³ g/ml, n=15; ◼, 10⁻⁴ g/ml, n=13). Drug was incubated for 30 minutes before repeating the second concentration-response curve. Data are expressed as a percentage of the maximal response obtained in the first (control) concentration-response curve. Curves are drawn by connecting the adjacent points. Values are mean ± S.E.M. (indicated by vertical bars) from n experiments.

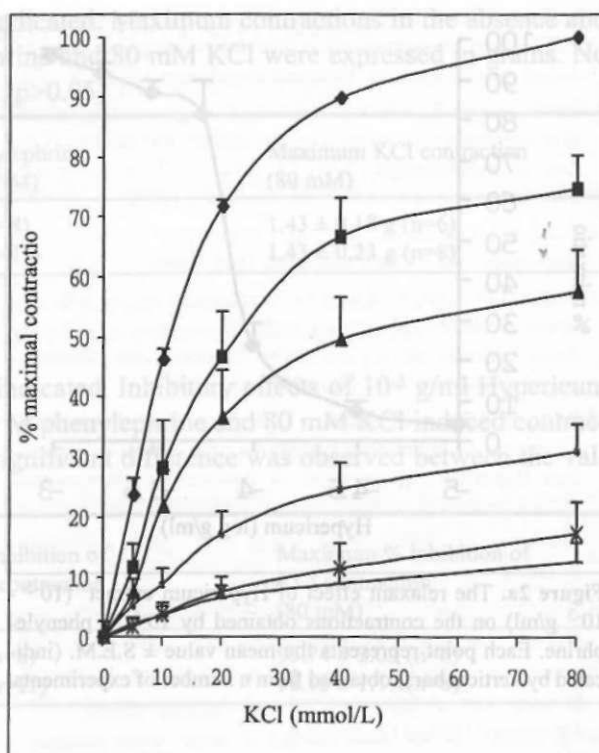


Figure 1b. Concentration-response curve of contractile responses of rat aorta for KCl in the absence (•, control, n = 47) and presence of Hypericum extract (◼, 10⁻³ g/ml, n = 9; ◼, 3 x 10⁻³ g/ml, n=9; ◻, 10⁻⁴ g/ml, n=11; ×, 3 x 10⁻⁴ g/ml, n=11; ○, 10⁻⁵ g/ml, n=7). Drug was incubated for 30 minutes before repeating the second concentration-response curve. Data are expressed as a percentage of the maximal response obtained in the first (control) concentration-response curve. Curves are drawn by connecting the adjacent points. Values are mean ± S.E.M. (indicated by vertical bars) from n experiments.

for contractions obtained with KCl. DMSO (4%) exerted no significant effect on the sustained contractions obtained with phenylephrine and KCl. The contractile effects of both agents were maintained without significant tension changes in control rings for at least 90 minutes.

(iii) Effect of Hypericum extract in the presence and absence of endothelium

Phenylephrine (10⁻⁶ M) and KCl (80 mM) produced sustained contractions in the rat isolated aortic rings with or without endothelium. No significant differences were observed between the maximal tensions reached (Table 1). Similarly, removal of endothelial layer did not alter the effect of Hypericum extract on phenylephrine and KCl induced contractions of rat aorta. There was no significant difference in inhibition by Hypericum ex-

tract of contractions in the absence and presence of endothelium (p>0.05) (Table 2).

Discussion

In the present study, the relaxant effect of *H. triquetrifolium* extract in response to phenylephrine and KCl induced contractions was investigated in rat isolated aorta. The results clearly demonstrated that the extract relaxed the precontracted rat aorta in a concentration-dependent fashion. It was also clear that this relaxant effect was independent of the presence of endothelium.

Hypericum extract reduced the maximal response to phenylephrine and KCl induced contractions, suggesting that it can be a non-competitive antagonist of α -adrenoceptors in aortic smooth

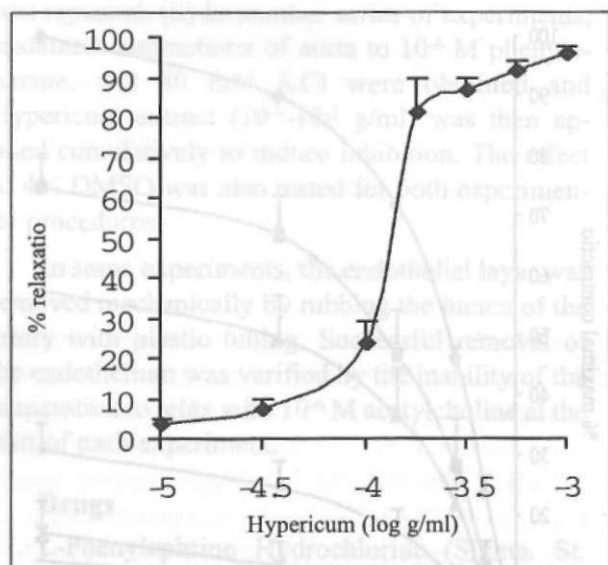


Figure 2a. The relaxant effect of Hypericum extract (10^{10} - 10^{10} g/ml) on the contractions obtained by 10^{10} M phenylephrine. Each point represents the mean value \pm S.E.M. (indicated by vertical bars) obtained from n number of experiments.

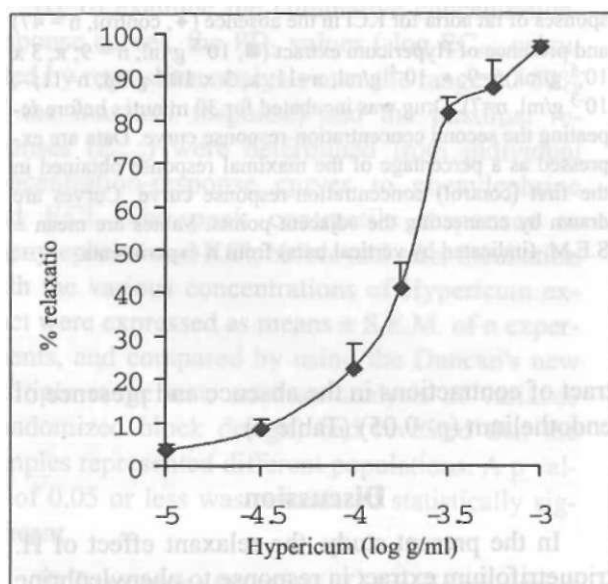


Figure 2b. The relaxant effect of Hypericum extract (10^{10} - 10^{10} g/ml) on the contractions obtained by 80 mM KCl. Each point represents the mean value \pm S.E.M (indicated by vertical bars) obtained from n number of experiments.

muscle (15). However, since it had been shown by other studies that the affinity of this plant extract for α -adrenoceptors were rather low (16) this rai-

ses a possibility that it must act at sites other than these receptors in smooth muscle.

As it was mentioned before the plant extract, concentration dependently inhibited the KCl induced contractions of isolated rat aorta in our study. It has been reported that KCl causes smooth muscle contraction by depolarizing cell membranes and by increasing the influx of Ca^{2+} through L and T voltage-dependent Ca^{2+} channels which in turn may induce CICR (Ca^{2+} -induced Ca^{2+} release) from intracellular stores (17-20). Therefore, extracellular Ca^{2+} entry is thought to be the major cause of KCl induced contractions. These findings indicate that Hypericum extract could inhibit Ca^{2+} entry through the depolarized cell membrane. Since the plant extract shares the same mechanism of action with tricyclic antidepressants, this supports the early reporting of antagonism between Ca^{2+} and tricyclic antidepressant drugs (15).

Moreover, it was clearly shown in this study that Hypericum extract inhibited phenylephrine induced contractions of rat aorta. It is generally believed that phenylephrine and noradrenaline (NA) induced contractions are dependent on the release of Ca^{2+} from NA-sensitive intracellular stores and Ca^{2+} influx through dihydropyridine-insensitive receptor operated Ca^{2+} channels (18, 21, 22). This results in elevated cytoplasmic Ca^{2+} levels and protein kinase C (PKC) is involved in promoting Ca^{2+} entry through channels. Both inorganic Ca^{2+} channel blockers and PKC inhibitors inhibit tonic contractions in response to NA in rat aorta (23). Since Hypericum extract caused a concentration-dependent decrease on phenylephrine-induced contractions, it can be stated that PKC-mediated steps in excitation-contraction coupling may be the site of action for the extract.

In the present work, Hypericum extract inhibited the phenylephrine and KCl-induced contractions in a concentration-dependent fashion and with almost equal effectiveness in rat aortic rings. Since it has been already demonstrated that the extract had no affinity on α -adrenoceptors, it can be suggested that the vasorelaxant effect of the extract may be due to an inhibition of Ca^{2+} entry in cell membranes.

The presence of the endothelium did not significantly modify the vasorelaxant effects of

Table 1. Values are means ± S.E.M. of n experiments indicated. Maximum contractions in the absence and presence of endothelium obtained by 10⁻⁶ M phenylephrine and 80 mM KCl were expressed in grams. No significant difference was observed between the values (p>0.05)

	Maximum phenylephrine contraction (10 ⁻⁶ M)	Maximum KCl contraction (80 mM)
With endothelium	1.40 ± 0.21 g (n=8)	1.43 ± 0.18 g (n=6)
Without endothelium	1.42 ± 0.15 g (n=11)	1.43 ± 0.23 g (n=8)

Table 2. Values are means ± S.E.M. of n experiments indicated. Inhibitory effects of 10⁻³ g/ml Hypericum extract were expressed as percentage inhibitions of 10⁻⁶ M phenylephrine and 80 mM KCl induced contractions in the absence and presence of endothelium. No significant difference was observed between the values (p>0.05)

	Maximum % inhibition of phenylephrine contraction (10 ⁻⁶ M)	Maximum % inhibition of KCl contraction (80 mM)
With endothelium	99.15 ± 0.55 (n=8)	95.74 ± 2.03 (n=6)
Without endothelium	94.67 ± 1.52 (n=11)	98.18 ± 1.19 (n=8)

Hypericum extract. This indicates that this effect is not due to an indirect and / or a direct action of the drug on the endothelium by increasing the release of endogenous vasodilator substances from endothelial cells and / or inhibiting the production of endothelium-derived contracting factors (24). Although the relaxant activity of H. triquetrifolium extract on rat aortic smooth muscle preparations was evident, the mechanism of this vasodilatory effect have not been definitely stated.

Conclusions

The present results demonstrate that high concentrations of total extract of H. triquetrifolium antagonized the contractile activity in the rat aortic smooth muscle preparations. Inhibition of Ca²⁺ entry through voltage and receptor-gated channels and of Ca²⁺ release from NA-sensitive intracellular stores or inhibition of the PKC-mediated contractile mechanism leading to a decrease in the availability of Ca²⁺ required for activation may contribute to this antagonism. Further studies must be conducted in order to clarify the exact mechanism of the vasorelaxant effect of this plant extract and to figure out which constituent of the extract mainly exerts this activity. In vivo controlled studies must

also be conducted in order to draw a conclusion in humans.

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