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RESEARCH ARTICLE

Contractile Effects of *Eryngium kotschyi* Boiss. on Rat Isolated Ileum and Detrussor Muscle [1]

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Summary

The pharmacological activity of the aerial (EKA) and root (EKR) parts of the endemic plant, *Eryngium kotschyi* Boiss., on rat isolated ileum and detrusor muscle was investigated. Plant extracts alone and with the presence of agonist (acethylcholine) and antagonist (atropin, verapamile, oxybutinine-detrusor muscle, and papaverine-ileum) drugs, along with Ca^{2+} applications on calcium-free medium, were applied. Plant extracts induced contraction in ileum and detrusor muscle where the contractions were concentration dependant for EKA and EKR single dose applications in detrusor muscle and concentration-free contractions were observed in cumulative applications for both tissues. Aerial and root parts of Eryngium extracts induced contractions in dose, tissue and protocol dependent manner where the contractions were affected by the tested antagonists, which could be attributed to non-specific pathways including calcium ions and calcium channel stimulations.

Keywords: *Eryngium kotschyi, Detrusor muscle, Bladder, Ileum, Motility, Rat*

Eryngium kotschyi Boiss.'in İzole Rat İleum ve İdrar Kesesi Düz Kasında Kastırıcı Etkisi

Özet

Bu çalışmada, ülkemizdeki endemik bitkilerden *Eryngium kotschyi* Boiss.'in toprak altı (EKTA) ve toprak üstü (EKTU) kısımlarının izole sıçan ileum ve idrar kesesi kasında farmakolojik etkinliği araştırıldı. Bitki ekstrelerinin dokulardaki etkinliği tek, agonist (asetilkolin) ve antagonist (atropin, verapamil, oksibutinin-idrar kesesi, papaverin-ileum) varlığında ve kalsiyumsuz ortamda Ca^{2+} uygulamaları ile birlikte değerlendirildi. Bitkinin her iki kısmı doku türü, ekstre dozu ve uygulama protokolüne bağlı değişiklik gösterecek şekilde kontraksiyon oluştururken; bu kasılmaların EKTU ve EKTA tek uygulamalarında doza bağımlı, kümülatif uygulamalarında ise dozdan bağımsız olduğu görüldü. Oluşan kasılmaların test edilen antagonistler ile değiştiği; dolayısıyla kontraktil etkinliğin kalsiyum iyonu ve kalsiyum kanallarının uyarılması gibi nonspesifik yolaklara özellikle bağlı olabileceği görüşüne varıldı.

Anahtar sözcükler: *Eryngium kotschyi, İdrar kesesi, İleum, Düz kas, Motilite, Sıçan*

INTRODUCTION

Medicinal plants and products thereof are doubtlessly of great medicinal and economic importance [1]. Since herbal medicines are considered to be safe and effective and regarded as free from undesirable side effects; many people turn to use instead of conventional drug therapy, globally [2].

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The genus *Eryngium*, belonging to the subfamily Saniculoidea of Apiaceae, are represented by 317 accepted taxa worldwide [3]. These species are named as "Boğadikeni" in Turkish folk medicine and are widely distributed around the country. *Eryngium kotschyi* named as "Çakır dikeni" is recorded as one of the ten endemic *Eryngium* taxa in Turkey [4]; however, ethnopharmacological use of *E. kotschyi* have not been reported yet. Detailed phytochemical investigation on *E. kotschyi,* resulted in isolation of four triterpenoid saponins [5]. Some Eryngium subspecies are used as vegetables or for sweetmeats in the Eastern Anatolia and assumed as cultural ornaments. On the other hand, infusions of the aerial and the root parts of some are used in folk medicine for its antitussive, diuretic, antiedema, appetizing, spasmolytic, stimulant, carminative and aphrodisiac effects and particularly used for urinary system disorders such as uremia and nephritis [6-10]. Related to the ethnopharmacological use of *Eryngium* species, especially as diuretic and spasmolytic [11,12], this study was based on investigating the potential effects of these plants on ileum and vesica urinaria *in vitro*. Therefore, the aim of this study was to investigate the pharmacodynamic effects of aerial and root parts of the promising endemic plant, *E. kotschyi,* on rat isolated ileal and detrusor muscle to gather *in vitro* data for further *in vivo* follow-up studies.

MATERIAL and METHODS

Preparation of Plant Extracts

E. kotschyi was collected during flowering time from Konya, Hadim-Beysehir Lake (1.500 m) in August 2009; authenticated by Prof. Dr. Hayri Duman (Gazi University Faculty of Arts and Sciences); given a voucher specimen and stored in Hayri Duman Herbarium (H. Duman - 9137). The root and the aerial parts of the plant were seperated (*E. kotschyi* aerial - EKA, *E. kotschyi* root - EKR) and dried in a cool-dark place and powdered using pulverizing mill. The powdered material was then extracted under reflux. The extract filtered and stored at -80°C [13]. The frozen extract was then lyophilized. The desired concentrations (w/v) were prepared from this extract for the analysis.

Animals

Thirty six male Wistar albino rats of 5 months weighing 250-350 g, were obtained from Ankara University, Animal Experimentation Unit (Ethical Approval: 2009-51-261). The animals were fasted overnight (had free access to water) and anesthetized with ether and sacrificed by decapitation.

Tissue Preparation

Detrusor smooth muscle (vesica urinaria) was removed and immersed in Krebs (in mM: NaCl 118, KCl 4.6, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 10, EDTA 0.025, pH 7.4) physiological solution; where as ileum was mounted in Tyrode (in mM: NaCl 136.89, KCl 2.68, $MgCl_2$ 1.05, $CaCl_2$ 1.80, NaH_2PO_4 0.42, $NaHCO_3$ 11.90, glucose 5.5, pH 7.4) physiological solution. Both tissues were cleaned from the surrounding connective tissue and cut into the strips of 1.5 cm length and then, gently suspended and isomerically connected to an isomeric force transducer (MAY-COM FDT 10-A, Commat Iletisim Ltd. Ankara, Turkey) on 10 ml organ baths filled with physiological solution. The baths were aerated with 95% O_2, 5% CO_2 at 37°C under a resting tension of 1.000 mg and allowed to equilibrate for 60 min in physiological solution [14]. The measurement of isometric force was continuously displayed and recorded on-line on a personal computer via a data acquisition system (TDA 94, Commat Iletisim Ltd.) using a software (Polywin 95, Ver 1.0; Commat Iletisim Ltd., Turkey) which also had the capacity to analyze the data.

Drugs

Lyophilized aqueous extracts of both the aerial and root parts of *E. kotschyi* were dissolved in water at the concentration of 150 mg/ml, and further dilutions were done accordingly and added to 10 ml Krebs-Tyrode containing organ bath as 100 µl where the concentrations were calculated as 100 folds. Papaverine hydrochloride, oxybutynin chloride, atropine sulfate, verapamil hydrochloride, and acetylcholine (ACh) (all chemicals were purchased from Sigma, St. Louis, MO, USA) were dissolved in water (Milli-Q double distilled) as 100 mM stock solutions and further diluted as required. Calcium chloride (Sigma-Aldrich, USA) were dissolved and diluted in Tyrode solution for ileal and Krebs solution for detrusor muscle applications.

Experimental Design

After a stabilization period of 60 min, contractile responses were recorded by stimulating the tissues with the EC_{50} value of ACh (10^{-6} M). All tissues were then given a 30 min equilibration period during which they were washed and the resting tension was adjusted in every 15 min.

***Single and cumulative dose administration of EKA and EKR to detrusor muscle and ileum*:** EKA was studied at 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 75, 100, 150 mg/ml single doses in detrusor muscle. The dose response curve was then assessed by 12.5, 25, 50, 75, 100, 150 mg/ml single doses where the response was found to be more accurate. Cumulative applications of EKA were carried out by 3.125, 6.25, 12.5, 25, 50, 75, 100, 150 mg/ml. EKR were studied at 25, 50, 75, 100 mg/ml for single doses and 12.5, 18.75, 25, 37.5 mg/ml for cumulative doses. EKA was studied at 0.078, 0.156, 0.3125, 0.78, 1.56 mg/ml single doses in ileum. The dose response curve was then assessed by 0.156, 0.3125, 0.78, 1.56 mg/ml single doses where the response was found to be more accurate. Cumulative applications of EKA were carried out by 0.078, 0.156, 0.3125, 0.78, 1.56, 3.125, 6.25 mg/ml. EKR were studied at 25, 50, 75, 100 mg/ml for single doses and 12.5, 18.75, 25, 37.5 mg/ml for cumulative doses.

***Cumulative and single dose of ACh administration to detrusor muscle and ileum after single dose incubation of EKA and EKR*:** After 10 min incubation of the muscle strips by the working doses close to EC_{50} for EKA (75 mg/ml) and EKR (50 mg/ml) ACh in cumulative doses (0.5 log folds of 10^{-8}-10^{-3} M) were applied. Same protocol was repeated by single dose application of ACh (10^{-6} M). After 10 min incubation of the muscle strips by the working doses close to EC_{50} for EKA (2.343 mg/ml) and the working dose close to E_{max} value (50 mg/ml) for EKR, ACh in cumulative doses (0.5 log folds of 10^{-8}-10^{-3} M) were applied. Same protocol was repeated by single dose application of ACh (10^{-6} M).

***Single dose of EKA and ACh administration to detrusor muscle and ileum followed by single dose of atropine, verapamil, oxybutynin, and papaverine incubation*:** EKA single dose (75 mg/ml) and ACh single dose (10^{-6} M) were administred to detrusor muscle and the results were recorded. After washing and equilibration, EKA single dose (75 mg/ml) and ACh single dose (10^{-6} M) were administered followed by 10 min incubation of antagonist drugs atropine (10^{-6} M) [15], verapamil (10^{-7} M) [15], and oxybutynin (10^{-8} M) [16]. EKA single dose (2.343 mg/ml) and ACh single dose (10^{-6} M) were administred to ileum and the results were recorded. After washing and equilibration, EKA single dose (2.343 mg/ml) and ACh single dose (10^{-6} M) were administered followed by 10 min incubation of antagonist drugs atropine (10^{-6} M) (7), verapamil (10^{-7} M) and papaverine (10^{-6} M) [17].

***Single dose of EKR and ACh administration to detrusor muscle and ileum followed by single dose of atropine, verapamil, oxybutynin, and papaverine incubation*:** EKR working dose (50 mg/ml) and ACh single dose (10^{-6} M) were administred to detrusor muscle and the results were recorded. After washing and equilibration, EKR single dose (50 mg/ml) and ACh single dose (10^{-6} M) were administered followed by 10 min incubation of antagonist drugs atropine, verapamil, and oxybutynin. EKR working dose (50 mg/ml) and ACh single dose (10^{-6} M) were administred to ileum and the results were recorded. After washing and equilibration, EKR single dose (50 mg/ml) and ACh single dose (10^{-6} M) were administered followed by 10 min incubation of antagonist drugs atropine, verapamil and papaverine.

***$CaCl_2$ administration, followed by the single dose incubation of EKA and EKR with or without the presence of verapamil to the detrusor muscle and ileum*:** Responses of the detrusor muscle to 1 mM $CaCl_2$ were recorded in calcium-free Krebs solution. After washing and equilibration, working doses of EKA (75 mg/ml), EKR (50 mg/ml) with and without the presence of verapamil (10^{-7} M) were administered, results were recorded. Following washing and equilibration, 1 mM $CaCl_2$ were applied after incubation with the plant extracts for 10 min and the responses were recorded. Responses of the ileum to 1 mM $CaCl_2$ were recorded in calcium-free Tyrode solution. After washing and equilibration, working doses of EKA (2.343 mg/ml), EKR (50 mg/ml) with and without the presence of verapamil (10^{-7} M) were administered, results were recorded. Following washing and equilibration, 1 mM $CaCl_2$ were applied after incubation with the plant extracts for 10 min and the responses were recorded.

Statistical Analysis

The results were presented as the mean±SEM of *n* observations. The contraction responses were expressed as apparent affinity constant (pD_2) and percentage of the corresponding maximal responses to the plant extract were calculated as a percentage of the maximal response to Ach (E_{max}). pD2 value was given by the negative logarithm of the molar agonist concentration that produces 50% of the maximal response produced by ACh (pD_2 = –logEC50). Values were analysed using Student's *t*-test or Mann Whitney U test, as appropriate after checking normality with Shapiro-Wilk and homogeneity of variances with Levene test as parametric test assumptions. Minimum of 5% of significance were considered to differ significantly. pD_2, EC_{50}, E_{max} values were calculated by interpolation from semilogarithmic plots by GraphPad Prism® and all statistical analysis were calculated using SPSS® 14.01 for Windows.

RESULTS

***Single and cumulative dose administration of EKA and EKR to detrusor muscle*:** pD_2 values for single dose and cumulative dose applications of EKA were calculated as 1.283±0.42 and 1.276±0.26, with the EC_{50} values of 52.13 and 53.02. E_{max} values for single dose applications were found at the highest dose (150 mg/ml) as 91.35±8.50 and for cumulative applications as 55.38±10.89 since downregulation were recorded at the highest dose for EKA *(Fig. 1)*. 75 mg/ml for EKA was decided as the working dose for detrusor muscle tissue giving precise results for both single and cumulative dose applications in accordance with the calculated EC_{50}. Dose dependant contractile response was observed by single dose administration of EKR in 25, 50, 75, 100 mg/ml single doses where EC_{50}, E_{max} and pD_2 were calculated as 48.81, 89.45±5.86 and 1.31±0.68, respectively. Highest tension was recorded at 150 mg/ml dose application as 1748±133.03 mg. Further protocols were carried out by 50 mg/ml close to the calculated EC_{50} (48.81) *(Fig. 2)*.

Single and cumulative dose administration of EKA and EKR to ileum: Single dose applications of EKA to ileum induced slight contractility in the muscle; however this contractility was not found dose-dependant. Highest tension was recorded at 0.078 mg/ml dose application as 1591±79.94 mg. In cumulative dose applications, EKA induced tension at lower doses; however this response was not dose-dependant where the responses decreased after 6.25 mg/ml. Single dose applications of EKR to ileum induced contractility in the muscle free from the

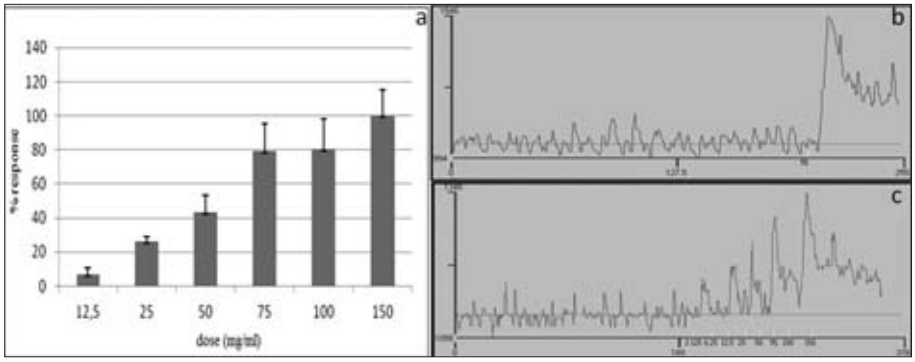

Fig 1. Single **(a,b)** and cumulative **(c)** dose responses of *Eryngium kotschyi* aerial in detrusor muscle

Şekil 1. İdrar kesesinde *Eryngium kotschyi* toprak üstü tek **(a,b)** ve kümülatif doz **(c)** cevapları

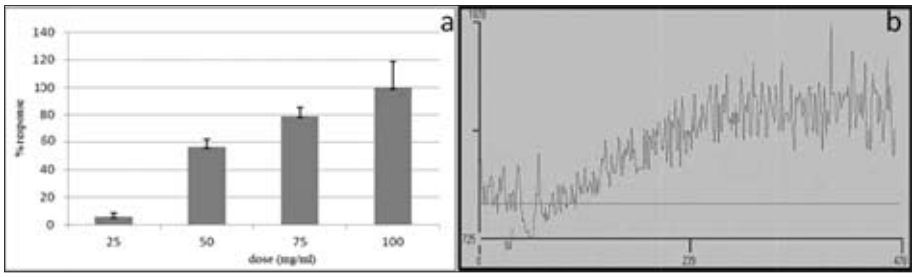

Fig 2. Single dose response of *Eryngium kotschyi* root in detrusor muscle

Şekil 2. İdrar kesesinde *Eryngium kotschyi* toprak altı tek doz cevapları

dose (except 25 and 50 mg/ml) giving long duration of contractility (≈7 min). Highest tension was recorded at 50 mg/ml dose application as 2315±110.93 mg where the working dose was chosen accordingly. Noncumulative contractility was observed until 75 mg/ml with the down-regulation at further doses.

Cumulative and single dose of ACh administration to detrusor muscle and ileum after single dose incubation of EKA and EKR: Isolated detrusor showed 30.14% contractile activity with the incubation of EKA (75 mg/ml) alone compared to single dose ACh (10^{-6} M) contraction alone. By the addition of ACh (10^{-6} M) over the EKA incubated bath, contractile responses increased to 144.78% (P<0.05). Similar results were obtained by EKR incubation as 29.85% EKR alone and 154.12% by ACh addition *(Table 1)*. Dose-response relation was not observed in cumulative applications. Isolated ileum showed 14.29% contractile activity with the incubation of EKA (2.343 mg/ml) alone compared to single dose ACh (10^{-6} M) contraction alone. By the addition of ACh (10^{-6} M) over the EKA incubated bath, contractile responses increased to 105.24% (P<0.05). EKR incubation (50 mg/ml) induced as 20.95% contraction alone and 77.14% by ACh addition *(Table 1)*. Dose-response relation was not observed in cumulative applications.

Single dose of EKA and ACh administration to detrusor muscle and ileum followed by single dose atropine, verapamil, oxybutynin, and papaverine incubation: Induced contractility in detrusor tissue by the incubation of EKA (75 mg/ml) alone according to ACh (10^{-6} M) contraction alone as 24.07% was decreased by the presence of atropine, verapamil, oxybutynin as 9.10%, 7.01%, 10.63%, respectively. No significant difference between different antagonists by the presence of the plant extract. ACh

Table 1. *Single dose responses of acethylcholine (ACh; 10^{-6} M) over Eryngium kotschyi aerial (EKA; 75 mg/ml for detrusor muscle and 2.343 mg/ml for ileum) and Eryngium kotschyi root (EKR; 50 mg/ml) incubation*

Tablo 1. *İdrar kesesinin ve ileumun Eryngium kotschyi toprak üstü (2.343 mg/ml) ve Eryngium kotschyi toprak altı (50 mg/ml) tek doz uygulaması üzerine tek doz asetilkolin (ACh; 10^{-6} M) cevapları*

Application	Response (%)			
	Detrusor muscle	P	Ileum	P
ACh (n: 4)	100±24.08		100±27.34	
EKA (n: 4) EKA + ACh (n: 4)	30.14±3.86[a] 144.78±23.52[b]	*	14.29±4.27[a] 105.24±32.86[b]	*
EKR (n: 3) EKR + ACh (n: 4)	29.85±3.16[a] 154.12±25.81[b]	*	20.95±5.44[a] 77.14±24.85[b]	*

*Different superscript letters ([a,b]) in a column for EKA and EKR groups, shows statistically significant difference represented by * for P<0.05*

(10^{-6} M) response to the same antagonists were found as 6.42%, 37.45%, 17.83%, respectively *(Table 2)*. EKA was found to be affected by the antagonists as ACh. Contractile responses (9.01%) of EKA in ileum were decreased by the presence of atropine, verapamil and papaverine (2.96, 3.38, and 3.18%, respectively). Whereas ACh administration with the presence of the antagonist drugs are found as 5.14%, 51.52%, and 77.39%, respectively where significant difference was observed compared to the EKA applications (P<0.05) *(Table 2)*.

Single dose of EKR and ACh administration to detrusor muscle and ileum followed by single dose atropine, verapamil, oxybutynin, and papaverine incubation: Contractile responses of EKR (29.37%) in detrusor muscle was decreased by the presence of atropine, verapamil, and oxybutynine (13.79%, 2.86%, and 6.57%, respectively). Verapamil response was found significantly different than

the other antagonist drugs for EKR applications (P<0.05). ACh responses with the presence of the antagonist drugs are found as 6.48%, 30.84%, and 12.22%, respectively where significant difference was observed compared to the EKR applications (P<0.05) *(Table 3)*. Contractile responses of EKR (33.54%) in ileum was decreased by the presence of atropine, verapamil and papaverine (19.27%, 14.11%, and 10.18%, respectively) without significant difference in between (P>0.05). ACh responses for the same antagonists were recorded as follows, 6.55%, 52.44%, and 83.97%, respectively with a significant difference between the EKR extracts by the contraction (P<0.05). Atropine, decreased the contractile responses of both ACh and EKR in ileum, whereas the results were found significant in ACh (P<0.05). On the other hand, EKR was more effected by papaverine and verapamil compared to ACh group *(Table 3)*.

$CaCl_2$ administration, followed by the single dose incubation of EKA and EKR with or without the presence of verapamil to the detrusor muscle and ileum: 1 mM $CaCl_2$ induced 24.29 mg tension difference in calcium-free Krebs solution; whereas EKA (75 mg/ml) induced 5.00±1.58 and EKR (50 mg/ml) induced 9.17±3.96 tension difference in detrusor muscle. After single dose incubation of EKA (75 mg/ml), EKR (50 mg/ml) and verapamil (10^{-7} M) 1 mM $CaCl_2$ found to increse the responses of the plant extract as potentialization whereas it was not able to break the antagonist effect of verapamile *(Table 4)*. 1 mM $CaCl_2$ induced 50.22 mg tension difference in calcium-free Tyrode solution. After single dose incubation of EKA (2.343 mg/ml), EKR (50 mg/ml) and verapamil (10^{-7} M); 1 mM $CaCl_2$ found to increse the responses of the plant extract as potentialization whereas it was not able to break the antagonist effect of verapamile *(Table 4)*.

DISCUSSION

For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas

Table 2. *Eryngium kotschyi aerial (EKA; 75 mg/ml for detrusor muscle and 2.343 mg/ml for ileum) and acethylcholine (ACh; 10^{-6} M) responses with atropine (10^{-6} M), verapamil (10^{-7} M), oxybutynin (10^{-8} M), and papaverine (10^{-6} M)*

Tablo 2. *İdrar kesesinin ve ileumun Eryngium kotschyi toprak üstü (idrar kesesi için 75 mg/ml, ileum için 2.343 mg/ml) tek doz uygulaması üzerine tek doz asetilkolin (ACh; 10^{-6} M), atropin (10^{-6} M), verapamil (10^{-7} M), oksibutinin (10^{-8} M) ve papaverin (10^{-6} M) cevapları*

Application	Response (%)			
	Detrusor muscle	P	Ileum	P
ACh (n: 5)	100±15.70		100±5.64	
EKA (n: 5)	24.07±3.93		9.01±1.59	
Atropine + EKA (n: 6) Atropine + ACh (n: 5)	9.10±4.46 [a] 6.42±2.51 [b]	*	2.96±0.88 [a] 5.14±1.98 [b]	*
Verapamil + EKA (n: 5) Verapamil+ ACh (n: 5)	7.01±2.01 [a] 37.4±6.74 [b]	*	3.38±2.18 [a] 51.52±5.99 [b]	*
Oxybutynin + EKA (n: 4) Oxybutynin + ACh (n: 4)	10.63±1.04 [a] 17.83±6.97 [b]	*	- -	
Papaverine + EKA (n: 5) Papaverine + ACh (n: 7)	- -		3.18±1.99 [a] 77.39±6.63 [b]	**

*Different superscript letters ([a,b]) in a column, shows statistically significant difference represented by * for P<0.05 and ** for P<0.01*

Table 3. *Eryngium kotschyi root (EKR; 50 mg/ml) and acethylcholine (ACh; 10^{-6} M) responses with atropine (10^{-6} M), verapamil (10^{-7} M), oxybutynin (10^{-8} M), and papaverine (10^{-6} M)*

Tablo 3. *İdrar kesesinin ve ileumun Eryngium kotschyi toprak altı (idrar kesesi ve ileum için 50 mg/ml) tek doz uygulaması üzerine tek doz asetilkolin (ACh; 10^{-6} M), atropin (10^{-6} M), verapamil (10^{-7} M), oksibutinin (10^{-8} M) ve papaverin (10^{-6} M) cevapları*

Application	Response (%)			
	Detrusor muscle	P	Ileum	P
Ach (n: 6)	100±7.87		100±7.76	
EKR (n: 4)	29.37±2.89		33.54±6.73	
Atropine + EKR (n: 4) Atropine + ACh (n: 6)	13.79±4.91 [a] 6.48±3.12 [b]	*	19.27±3.97 [a] 6.55±3.69 [b]	*
Verapamil + EKR (n: 6) Verapamil+ ACh (n: 5)	2.86±1.43 [a] 30.84±3.99 [b]	*	14.11±3.11 [a] 52.44±7.18 [b]	*
Oxybutynin + EKR (n: 6) Oxybutynin + ACh (n: 4)	6.57±2.08 [a] 12.22±4.78 [b]	*	-	
Papaverine + EKR (n: 4) Papaverine + ACh (n: 5)	-		10.18±2.04 [a] 83.97±10.50 [b]	*

*Different superscript letters ([a,b]) in a column, shows statistically significant difference represented by * for P<0.05*

Table 4. *Responses of Eryngium kotschyi aerial (EKA; 75 mg/ml for detrusor muscle and 2.343 mg/ml for ileum), Eryngium kotschyi root (EKR; 50 mg/ml) and verapamil (10^{-7} M) incubation and coapplications with 1 mM $CaCl_2$*

Tablo 4. *Eryngium kotschyi toprak üstü (idrar kesesi için 75 mg/ml ve ileum için 2.343 mg/ml) ve Eryngium kotschyi toprak altı (idrar kesesi ve ileum için 50 mg/ml) ve veya verapamil (10^{-7} M) ile inkübasyonu takiben 1mM $CaCl_2$ cevapları*

Application	Tension Difference (mg)			
	Detrusor muscle	P	Ileum	P
EKA (n: 7)	5.00±1.58		-77.83±43.49	
EKR (n: 7)	9.17±3.96		171.67±33.30	
$CaCl_2$ (n: 7) EKA + verapamil + $CaCl_2$ (n: 6) EKR + verapamil + $CaCl_2$ (n: 7)	24.29±5.17 [a] 32.50±13.15 [a] 135.71±26.89 [b]	* * *	50.22±11.27 [a] 165.00±28.36 [b] 285.00±71.20 [c]	* * *

*Different superscript letters ([a,b,c]) in a column, shows statistically significant difference represented by * for P<0.05*

of phytochemistry, pharmacognosy, and horticulture. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. Since phytomedicines exert their beneficial effects through the interaction of multiple chemical compounds at the same time through single or multiple target sites; the pharmacological effect could not be attributed to the main active component but the synergistic action of several compounds [18]. Phytochemical studies on *Eryngium* species revealed that this genus contains mainly the phenolic compounds and terpenoids including triterpenoid saponins, monoterpene, sesquiterpenes, triterpenoids, flavonoids, coumarins, steroids, acetylenes and other compounds [11]. Antispasmodic activity of triterpenoid saponin "zygophylosides" from *Zygophylum gaetulum* [19] and "ginseng saponins" from *Panax ginseng* [20] on isolated guinea pig ileum were reported previously. On the contrary, for the current study, since contraction responses were recorded in ileal and detrusor muscle strips; it could be suggested that, other compounds in the extract might have masked the triterpenoid effect where antispasmodic activity was expected or the extract acts through a cholinergic mechanism with the role of calcium ions which was confirmed by the responses by the antagonists in both tissues. In several *in vitro* studies, the importance of the muscarinic receptor systems on the mechanism of action of plant extracts with the influence of Ca^{2+} were discussed in details. For instance, Hu et al.[21] studied M3 muscarinic receptor- and Ca^{2+} influx-mediated muscle contractions induced by croton oil in isolated rabbit jejenum. In the study of Elorriaga et al.[22] muscarinic receptor-induced phasic contractions in the rat ileum were depended on the release of internal Ca^{2+} entry from the extracellular space through voltage-dependent Ca^{2+} channels. Parry et al.[23] investigated papaverine-like relaxant effects of the aqueous extract of the root bark of *Heteromorpha trifoliata* on gastrointestinal smooth muscle strips where the mechanism of action were related to the prevention of the Ca^{2+} influx into the smooth muscle cells, inhibiton of the calcium-induced Ca^{2+} release mechanism, prevention of the release of calcium from the sarcoplasmic reticulum, or prevention of the binding of calcium to calmodulin [24]. Therefore, Ca^{2+} has a critical role on the effects of the muscarinic receptor antagonists such as papaverine and oxybutynine. In the current study, atropine, papaverine and verapamil induced inhibitory effect over plant extract contraction. Among these antagonists, verapamil induced inhibition by both extract coadministration contractions were found to be more preponderant compared to ACh induced contractions. To sum up, the contractile responses of EKR and EKA could be related to receptor mediation and by voltage dependant (L type) calcium channels where the contractility were found to be affected at various levels by tested antagonists revealing the importance of the Ca^{2+} for the mechanism of action.

Inhibitory responses of 1 mM $CaCl_2$ administration, followed by the single dose incubation of the plant extracts and verapamil to both tissues in calcium-free media with respect to ACh, showed that Ca^{2+} had a nonspecific role in the contractility by *Eryngium* extracts. The decrease of contractility by plant extracts in calcium-free media compared to calcium included media; increase of the contractility by addition of 1 mM $CaCl_2$ in calcium-free media and the responses of the plant extracts by ACh compared to verapamil incubation; strengthens the theory of Ca^{2+} mediated pathways for the contractile responses of the extracts [25].

In conclusion, aerial and root parts of *E. kotschyi* induced contractility on tissue-dose dependent manner where the contractions were affected by the tested antagonists. The mechanism of action could be related to non-specific pathways including calcium ions and calcium channel mediated pathways. The present study revealed the contractile responses of *E. kotschyi* on detrusor and ileal tissues which should be further investigated by *in vivo* studies for its promising pharmacological effects on motility along with complementary toxicity tests and more research studies should be encouraged on the pharmacological activity of the endemic plants in Turkey [26] to reveal a basis for their ethnomedicinal use.

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