Kafkas Univ Vet Fak Derg
26 (5): 697-704, 2020
DOI: 10.9775/kvfd.2020.24286

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

The Effect of Greater Celandine Active Ingredient Chelidonine on Isolated Rat Bladder and Trachea Smooth Muscles and Primary Lung and Kidney Cell Lines [1]

Emre ARSLANBAŞ [1,a] Haki KARA [2,b] Nergiz Hacer TURGUT [3,c] Hüseyin GÜNGÖR [2,d]
Halef Okan DOĞAN [4,e] Mustafa Ozan ATASOY [5,f] Alper Serhat KUMRU [2,g]

[1] *This work is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University under the project number V-055 (CÜBAP, Sivas, Turkey)*

[1] Pharmacology and Toxicology Department, Faculty of Veterinary Medicine, Atatürk University, TR-25240 Erzurum - TURKEY
[2] Pharmacology and Toxicology Department, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, TR-58140 Sivas - TURKEY
[3] Pharmacology Department, Faculty of Pharmacy, Izmir Kâtip Çelebi University, TR-35620 İzmir - TURKEY
[4] Medicinal Biochemistry Department, Faculty of Medicine, Sivas Cumhuriyet University, TR-58140 Sivas - TURKEY
[5] Department of Virology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, TR-58140 Sivas - TURKEY
ORCIDs: [a] 0000-0003-0030-7195; [b] 0000-0002-0295-0582; [c] 0000-0003-1700-2451; [d] 0000-0002-2506-3855; [e] 0000-0001-8738-0760
[f] 0000-0003-0096-6297; [g] 0000-0001-8462-4264

Article ID: KVFD-2020-24286 Received: 04.04.2020 Accepted: 03.08.2020 Published Online: 04.08.2020

How to Cite This Article

Arslanbaş E, Kara H, Turgut NH, Güngör H, Doğan HO, Atasoy MO, Kumru AS: The effect of greater celandine active ingredient chelidonine on isolated rat bladder and trachea smooth muscles and primary lung and kidney cell lines. *Kafkas Univ Vet Fak Derg,* 26 (5): 697-704, 2020. DOI: 10.9775/kvfd.2020.24286

Abstract

This study aims to explore the pharmacodynamics of chelidonine, the active ingredient in greater celandine *(Chelidonium majus* L.), on *in vitro* rat bladder and trachea tissue, and evaluate its cell protective effects on primary lung and kidney cell lines. The study was carried out via repeated applications of acetylcholine, atropine, verapamil and oxybutynin, alongside Ca^{++} in a calcium-free environment, on urinary bladder tissue, and repeated applications of acetylcholine, atropine, carbachol and mecamylamine on trachea tissue. At the same time, cell viability and catalase and superoxide dismutase activity was measured on primary cell lines obtained from lung and kidney tissue samples. The study has shown that chelidonine has a relaxant effect on bladder and trachea tissues, and it may be mentioned that this effect is produced via muscarinic receptors. In addition, chelidonin caused a statistically insignificant increase in cell viability in primary lung and kidney cell lines at increasing doses (1 and 4 µg/mL), but this increase remained at the control group level. In contrast, chelidonin caused a significant decrease in cell viability at the same cell lines at doses of 8 and 16 µg/mL. In conclusion, it is suggested that greater celandine, which is used in folk medicine, and its active ingredient chelidonine might have beneficial effects on asthma, urinary incontinence and other urinary tract and respiratory diseases among others.

Keywords: *Chelidonine, Urinary bladder, Trachea tissue, Smooth muscle, Cell culture, Rat*

Kırlangıç Otu Etkin Maddesi Chelidoninin İzole Rat İdrar Kesesi ve Trakea Düz Kasları ve Primer Akciğer ve Böbrek Hücre Hatları Üzerine Etkisi

Öz

Bu çalışmada, kırlangıç otu *(Chelidonium majus* L.) etkin maddesi chelidoninin *in vitro* rat idrar kesesi ve trakea dokusu üzerindeki farmakodinamik etkilerinin değerlendirilmesi ve primer akciğer ve böbrek hücre hatları üzerine hücre koruyucu etkilerinin araştırılması amaçlandı. Çalışma, idrar kesesi dokusunda tekrarlı yapılan asetilkolin, atropin, verapamil ve oksibutinin ile kalsiyumsuz ortamda Ca^{++} uygulamalarıyla, trakea dokusuna ise tekrarlı uygulamalarla asetilkolin, atropin, karbakol ve mekamilamin verilerek gerçekleştirildi. Aynı zamanda, alınan akciğer ve böbrek dokularından elde edilen primer hücre hatları üzerinde hücre canlılığı ölçümleri, katalaz ve süperoksit dismutaz enzim aktivitelerinin tayini yapıldı. Çalışma sonucunda, kırlangıç otu etkin maddesi chelidoninin idrar kesesi ve trakea dokuları üzerinde gevşetici etkisinin olduğu belirlendi ve chelidoninin bu etkisini muskarinik reseptörler üzerinden gösterdiğinden söz edilebilir. Ayrıca, chelidonin artan dozlarda (1 ve 4 µg/mL) primer akciğer ve böbrek hücre hatlarında hücre canlılığında istatistiksel olarak önemsiz bir artışa neden oldu, ancak bu artış kontrol grubu seviyesinde kaldı. Buna karşılık chelidonin aynı hücre hatlarında 8 ve 16 µg/mL dozlarında hücre canlılığında önemli bir düşüşe neden oldu. Sonuç olarak, halk arasında tıbbi amaçlarla kullanılan kırlangıç otunun ve etkin maddesi chelidonin astım, üriner inkontinans, idrar yolları ve solunum sistemi hastalıkları vb. durumlarda faydalı etkileri olabileceği görüşüne varıldı.

Anahtar sözcükler: *Chelidonin, İdrar kesesi, Trakea dokusu, Düz kas, Hücre kültürü, Rat*

Correspondence
+90 442 2317028
emre.arslanbas@atauni.edu.tr

INTRODUCTION

Greater celandine *(Chelidonium majus* L.) is an herbaceous perennial plant from the Papaveraceae family, containing colored benzophenanthridine alkaloids. The plant has a long history of use in the treatment of various illnesses, particularly in European traditional medicine as well as Chinese herbal medicine [1-3]. In Turkey, *C. majus*, known with the names swallowwort and hilaliye, is a common ingredient in folk medicine [4]. The healing properties of the plant are mainly associated with the high content of biologically active compounds, and it is used in herbal medicine against bronchitis, jaundice, warts, skin cancer, digestive system conditions and eye diseases as well as for protection of the liver [1,3-5]. In particular, the dried flower-bearing branches, infusion, latex, tincture, leaf sap and decoction of *C. majus* L. are used for medicinal purposes. Greater celandine has sedative, hypnotic, analgesic, anti-inflammatory, anti-microbial, antitumoral, antiviral and laxative properties [2,6,7].

Chelidonium majus L. contains several types of alkaloids, such as the benzylisoquinoline group, which includes benzophenanthridines (chelidonine, chelerythrine, iso-chelidonine, sanguinarine), as well as protoberberines (berberine, coptisine, dihydrocoptisine, stylopine) and protopines (protopine). From these main alkaloids, chelidonine, coptisine and berberine are generally obtained from the top parts of the plant, while sanguinarine and chelerythrine are derived from the roots [2,5]. Chelidonine displays anti-inflammatory, immunomodulator, analgesic, antimicrobial, antiviral and antifungal properties [8,9].

The number of studies that explore the pharmacodynamics of chelidonine is limited [10-13]. While there are studies that focused at the plant extract alone, there are discrepancies in the results of studies that explore plant extracts and active ingredients, depending on the selection of animal species and tissue types used in the study. At the same time, researchers have voiced the need to supplement existing studies with more in-depth ones that investigate not only the active ingredients, but also their mechanisms of action. On the other hand, cell culture studies have shown that chelidonine has cytotoxic, apoptotic, antiproliferative, etc. effects on various cancer cell lines (e.g. human leukemia cells CEM, MT-4, MOLT-4 and Jurkat, hepatocarcinoma HepG2, breast cancer cells MCF-7, pancreatic and colon cancer cell lines) [5,6,9,14,15]. However, studies are mainly focused on the anticancer effects of chelidonine. This study aims to explore the pharmacodynamics of chelidonine, the active ingredient in greater celandine, on *in vitro* bladder and trachea tissue, and evaluate its cell protective effects on primary lung and kidney cell lines.

MATERIAL and METHODS

Drugs

The active ingredient used in the study, chelidonine (54274) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, US) and used to create a 1 mg/mL stock solution in ultrapure water. The remainder of the agents, namely acetylcholine (A6625), verapamil (V4629), oxybutynin (O2881), carbachol (C4382) and thiazolyl blue tetrazolium bromide (M5655) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, US); penicillin/streptomycin/amphotericin B (450-115 EL) from Wisent Inc. (Canada); atropine (226680100) from Acros Organics (Belgium); mecamylamine (M202600) from Toronto Research Chemicals Inc. (Ontario, Canada); and calcium chloride ($CaCl_2$, 328757) from Carlo Erba Reagents S.A.S. (Italy). All agents used in the study were at analytical grade.

Animals

The study used 36 adult Wistar Albino rats of 6 to 9 months of age, each weighing 250±20 g, which were raised at Sivas Cumhuriyet University Laboratory Animals Department. The study was conducted with Sivas Cumhuriyet University Animal Experiments Local Ethics Board (CÜHADYEK) approval 65202830-050.04.04-49 dated April 6, 2016.

Pharmacodynamics Study

The animals used in the study were anesthetized before being euthanized via cervical dislocation. Immediately afterwards, the urinary bladder and trachea tissues were removed. Urinary bladders were taken out through an incision made at the abdominal area, taking care to prevent damage to the samples. Each isolated bladder was placed in Krebs solution (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) before creating tissue preparations in the form of strips with dimensions of 2 mm x 0.5 mm x 10 mm. These preparations were placed in an isolated organ bath at a temperature of 37°C, continuous aeration using a gas mix of 95% O_2-5% CO_2, and inside 5 mL of krebs solution [16,17]. Tracheal tissues were removed without delay through an incision made at the neck area of the animals. Isolated trachea samples were placed in Krebs solution (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; pH 7.4). Afterwards, tracheal rings of 4 to 5 mm in length, fixed using stainless steel rings, were hanged in an isolated organ bath inside of krebs solution [18]. Isometric smooth muscle movements of urinary bladder and tracheal tissues inside the isolated organ baths were monitored and recorded using a "force transducer" (Force Displacement Transducer-FDT 05, Commat İletişim Ltd., Turkey) and an "acquisition system" (MP150 Biopac System, Commat İletişim Ltd., Turkey). The tissue samples were placed under 1.000 mg of tension and given at least 1 h to acclimatize to the environment, during which the krebs solution was replaced every 15 min. Once they reached equilibrium, the bladder and trachea tissues were stimulated using acetylcholine at EC_{50} values (10^{-6} M and 10^{-5} M, respectively). Following the contraction response, krebs solution in the baths were replaced; the samples

were washed and left to reach equilibrium once again. Afterwards, the following protocols were followed [17-20].

Single and cumulative dose chelidonine applications to urinary bladder smooth muscle and trachea tissue: Chelidonine was applied to the urinary bladder smooth muscle in single (10^{-7}; 10^{-6}; 10^{-5}; 10^{-4}; 10^{-3} M) and cumulative doses (10^{-7}-10^{-3} M, at 3 min intervals) to identify the dosage with the highest response (n=7). The same method was applied to tracheal tissues using the same dosages (n=8). As it was observed that cumulative dose applications to tracheal tissue produced more pronounced results, it was decided to focus further on cumulative dosages.

Acetylcholine application to urinary bladder smooth muscle and tracheal tissue after incubation in chelidonine: Chelidonine was applied to the bladder tissues in study dose and to tracheal tissues in cumulative doses, followed by 20 min of incubation. Afterwards, both urinary bladder and tracheal tissues were treated with a single dose of acetylcholine (10^{-6} M and 10^{-5} M, respectively) (n=8). Acetylcholine was also applied to urinary bladder smooth muscles and tracheal tissues in cumulative doses (10^{-8}-10^{-3} M) to determine its effects (n=6).

Chelidonine application to urinary bladder after incubation in atropine, verapamil and oxybutynin: Chelidonine was applied to the tissues in study dose followed by the application of acetylcholine (10^{-6} M), and the results were recorded (n=6). After the tissue samples are washed and left to reach equilibrium, they were incubated in antagonist atropine (10^{-6} M), verapamil (10^{-7} M) and oxybutynin (10^{-7} M), followed by an application of chelidonine in study dose.

Chelidonine application to trachea tissue after incubation in atropine, carbachol and mecamylamine: Trachea tissues were treated with chelidonine in cumulative-dose followed by the application of acetylcholine (10^{-5} M), and the results were recorded (n=6). Tracheal tissue samples were washed and left to reach equilibrium after replacement of the krebs solution, and then incubated in atropine (10^{-6} M), carbachol (10^{-6} M) and mecamylamine (10^{-5} M), followed by a cumulative-dose application of chelidonine.

1mM $CaCl_2$ application to urinary bladder smooth muscle in non-calcium Krebs solution after incubation in chelidonine and verapamil: The tissue samples were placed in a non-calcium krebs solution and treated with 1 mM $CaCl_2$, and the results were recorded (n=5). Afterwards, the samples were washed and left to reach equilibrium after replacement of the krebs solution. The tissue samples were treated with chelidonine in study dose and verapamil (10^{-7} M), and after 10 min of incubation, 1 mM $CaCl_2$ was added without washing the samples, and the results were recorded.

Cell Culture Study

Primary cell lines were used in this study. Primary cell lines were obtained from the lung and kidney tissues taken from the laboratory rats used in the study, in accordance with the method reported by Freshney [21]. The cells were put into flasks (10^6 cells/mL). After being prepared for chelidonine application, cell lines were left for 24 h to reach equilibrium. At the end of this period the samples were treated. Using the stock solution, five different concentrations of chelidonine were prepared (1, 2, 4, 8 and 16 µg/mL) and applied to the samples [14]. The control group was applied ultrapure water in equal volume. The control group and chelidonine-applied cells were left to incubate for 24 h. After incubation, cell viability and antioxidant enzyme (catalase-CAT and superoxide dismutase-SOD) activity measurements were taken. Cell viability was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity analysis. In the MTT analysis, chelidonin (1-16 µg/mL) was applied to the cells planted in 96-well plates, with 10.000 cells per well, and after 24 h incubated, the plate was incubated with a mediocre 0.5 mg/mL MTT containing 5% CO_2 and 37°C for 4 h. At the end of the period, the MTT solution was removed and the cells were lized with dimethylsulfoxide (DMSO). The plate was then placed on an orbital shaker for 6 minutes and then absorbance at 570 nm was read using a microplate reader (spectrophotometer) [15]. In CAT and SOD enzyme activity assays, cells were planted in 24-well plates with 10^4 cells in each well and grown for 24 h, then concentrations of chelidonin 1-16 µg/mL were applied. The cells were incubated for 24 h and the CAT and SOD enzyme activity measurements were taken at the end of the period. The test kit for the measurement of CAT and SOD enzyme activities was used in line with the protocol recommended by the producer (Cayman Chemical Company, US).

Statistical Analysis

Results of the study were presented as mean and standard error of the mean (SEM). Contraction responses were expressed as apparent affinity constant (pD_2), with the pD_2 value was given as the negative logarithm of the molar agonist concentration that produces 50% of the maximum response produced by acetylcholine ($pD_2 = -\log EC_{50}$). Percentage of the corresponding maximal responses to the active ingredient were calculated as a percentage of the maximal response to acetylcholine (E_{max}). Mann-Whitney U test was utilized to assess the difference between the tissue samples. Statistical significance was set at $P<0.05$. pD_2, EC_{50}, E_{max} and IC_{50} values were calculated by interpolating the figures using GraphPad Prism (Version 8.2.0), while statistical analyses were made using SPSS (Version 23).

RESULTS

Pharmacodynamics Results

Single and cumulative dose chelidonine applications to urinary bladder smooth muscle and trachea tissue: It was observed that chelidonine has a relaxant effect

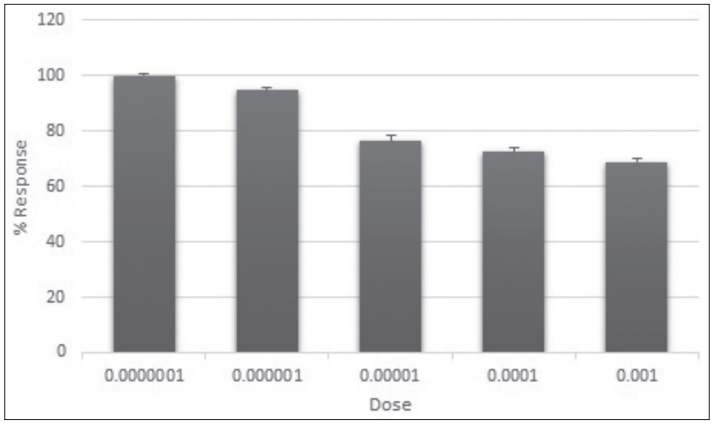
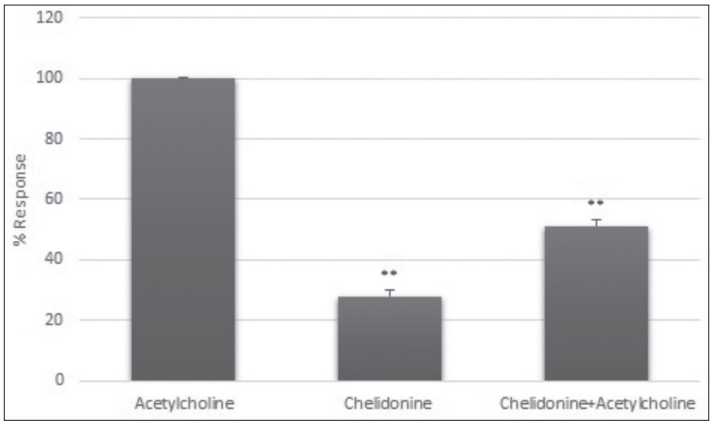


Fig 1. Single dose responses to chelidonine in urinary bladder

Fig 2. Relaxation responses of chelidonine and chelidonine + acetylcholine according to acetylcholine in the urinary bladder (** P<0.01)



on urinary bladder and trachea tissues, but the effect was not cumulative. In cumulative dose applications to urinary bladder and trachea tissues, pD_2 ($-logEC_{50}$) and E_{max} values were 5.024±0.24 and 101.5±2.74%; 4.870±0.01 and 100.7±0.09%, respectively. The chelidonine dose 10^{-5} M, which was shown to produce a better response based on the EC_{50} values obtained from single and cumulative dose applications to the urinary bladder, was selected for the study dose for the subsequent protocols. In single dose applications, % response for 10^{-5} M chelidonine dose was found to be 76.42±1.72% *(Fig. 1)*.

Acetylcholine application to urinary bladder smooth muscle and tracheal tissue after incubation in chelidonine: In urinary bladder tissues, 10^{-5} M dose of chelidonine induced a response of 27.96% by itself, and 51.16% when combined with acetylcholine (P<0.01) *(Fig. 2)*. Application of cumulative doses (10^{-7}-10^{-3} M) of chelidonine to trachea tissues followed by acetylcholine (10^{-5} M) resulted in an increase in contraction response; however, no correlation was observed between the dose and response. Application of cumulative doses of acetylcholine to bladder and trachea tissues provided pD_2 ($-logEC_{50}$) and E_{max} values of 3.718±0.24 and 120.0±16.29%; 3.672±0.52 and 119.8±15.79%, respectively.

Chelidonine application to urinary bladder after incubation in atropine, verapamil and oxybutynin: The contraction of the bladder on single-dose chelidonine (10^{-5} M) application (26.31%) showed a reduction in the presence of atropine (16.30%), verapamil (14.49%) and oxybutynin (10.92%) (P<0.01). Responses to acetylcholine (10^{-6} M) in the presence of the same antagonists showed a significant decline (13.25%, 74.40% and 37.13%, respectively), revealing a statistically significant difference from chelidonine responses (P<0.01) *(Fig. 3)*.

Chelidonine application to trachea tissue after incubation in atropine, carbachol and mecamylamine: It was noted that the presence of atropine, carbachol and mecamylamine affected the contraction of the trachea in response to cumulative doses of chelidonine (10^{-7}-10^{-3} M) (P>0.05). A statistically significant difference was observed between the responses to acetylcholine (10^{-6} M) in the presence of the same agents (14.54%, 62.66% and 40.39%, respectively) and chelidonine responses (P<0.01) *(Fig. 4)*.

1mM $CaCl_2$ application to urinary bladder smooth muscle in non-calcium Krebs solution after incubation in chelidonine and verapamil: The tension difference observed after incubation with 1 mM $CaCl_2$ in non-calcium krebs solution was 24.50 mg. Application of 1 mM $CaCl_2$ after single-dose chelidonine and verapamil incubation was shown to increase chelidonine response *(Fig. 5)*.

Cell Culture Results

MTT results: Cell viability was shown to be at a maximum in primary lung and kidney cells treated with 4 µg/mL

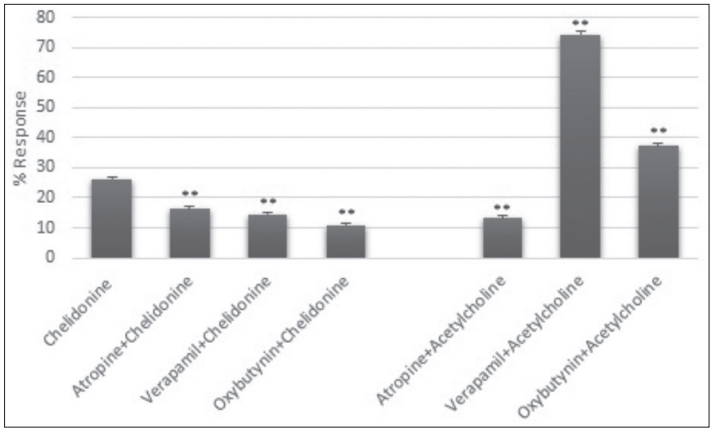
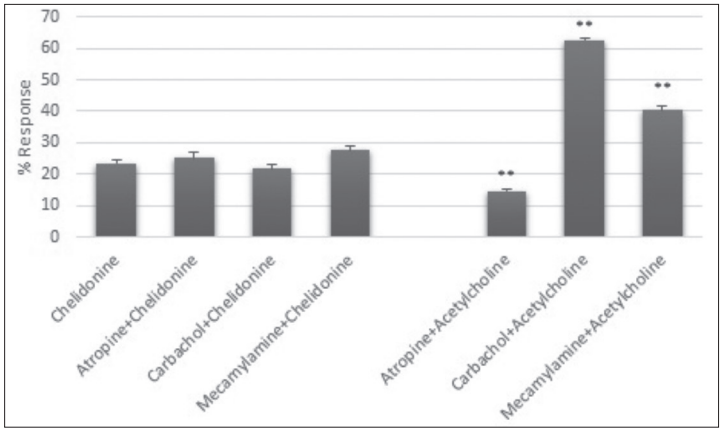


Fig 3. Antagonist+chelidonine and antagonist + acetylcholine relaxation responses in bladder (** P<0.01)

Fig 4. Agonist and antagonist chelidonine, agonist and antagonist acetylcholine relaxation responses in trachea tissue (** P<0.01)



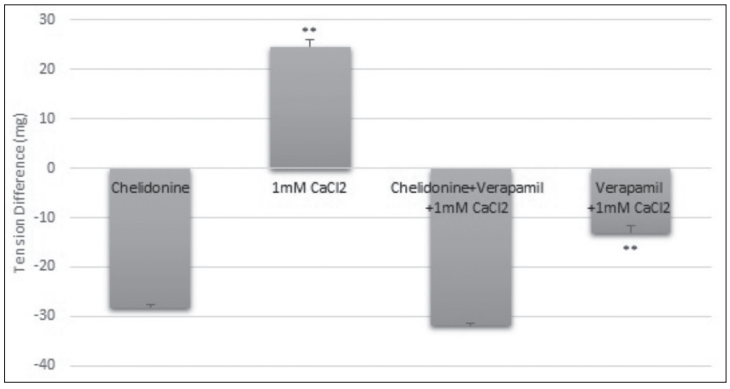


Fig 5. 1 mM $CaCl_2$ relaxation responses in bladder after chelidonine and verapamil (10^{-7} M) incubation in non-calcium krebs solution (** P<0.01)

of chelidonine, with further increases in concentration reducing cell viability *(Fig. 6)*. Chelidonine application revealed an IC_{50} value of 10.91 µg/mL in primary lung cells, and 12.19 µg/mL in primary kidney cells.

CAT and SOD results: Application of chelidonine in different concentrations (1-16 µg/mL) to primary lung and kidney cells revealed a concentration-based change in CAT and SOD levels in comparison to the control group. Application of chelidonine in a concentration of 4 µg/mL to primary lung cells (P<0.05) and in concentrations of 2 and 4 µg/mL to primary kidney cells (P<0.05) showed a statistically significant increase in CAT levels compared to control *(Fig. 7)*. Likewise, SOD levels displayed a statistically significant change from control group results when chelidonine was applied to primary lung cells in a concentration of 4 µg/mL (P<0.05) and to primary kidney cells in concentrations of 1, 2 and 4 µg/mL (P<0.05, P<0.01, P<0.05) *(Fig. 8)*.

DISCUSSION

The relationship between plants and humans is as old as history itself, and experiences passed down from generation to generation have resulted in a wealth of knowledge and treatment methods in time. It is necessary to identify the chemical composition and active ingredients, as well as their mechanisms of action, of these plants and herbs that are used in traditional medicine, in order to ensure proper, efficient and sufficient use [7,22].

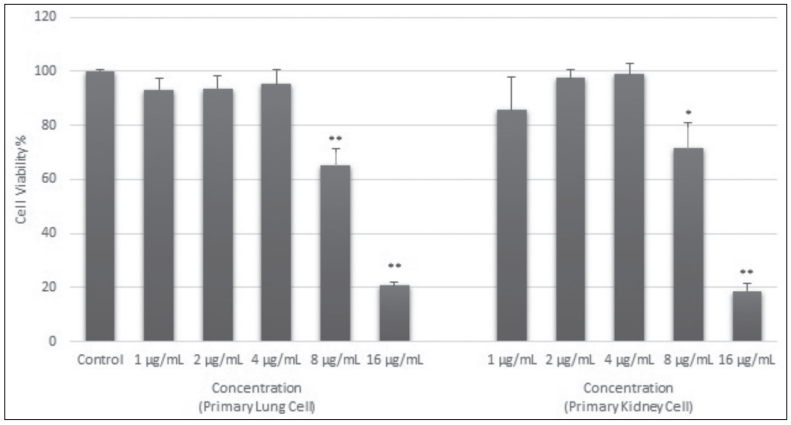
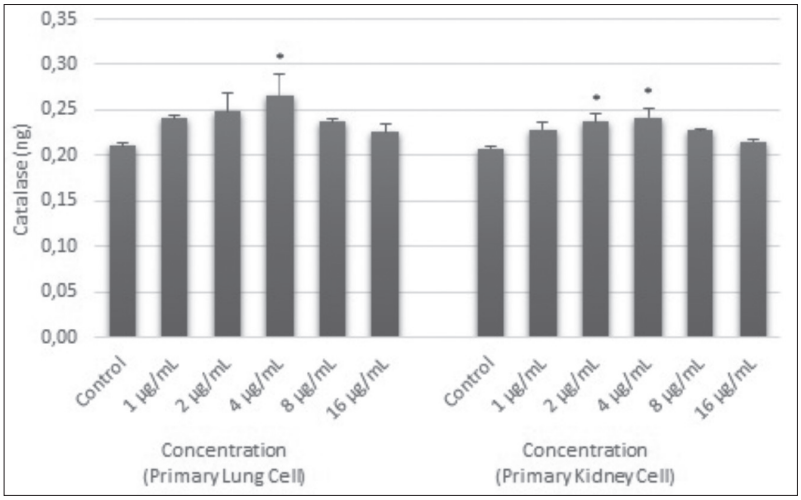


Fig 6. Cell viability % values of primary lung and kidney cells treated with chelidonine (* P<0.05; ** P<0.01)

Fig 7. Catalase values of primary lung and kidney cells treated with chelidonine (* P<0.05)



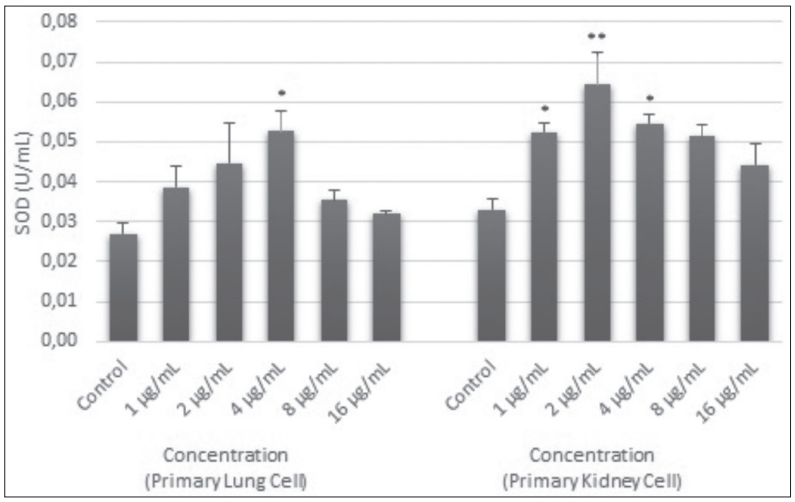


Fig 8. SOD values of primary lung and kidney cells treated with chelidonine (* P<0.05; ** P<0.01)

This study aimed to assess the pharmacodynamics of chelidonine, the active ingredient in greater celandine, a plant used in folk medicine against a variety of conditions, on *in vitro* rat bladder and trachea tissue, and to evaluate its cell protective effects on primary lung and kidney cell lines. The results of this study have shown that chelidonine has a relaxant effect on urinary bladder and trachea tissues *(Fig. 1)*. In a study by Hiller et al.[11], which explored the effects of the primary alkaloids derived from the ethanolic extract of greater celandine *(C. majus* L.), namely chelidonine, protopine and coptisine on guinea pig ileum tissue, it was shown that chelidonine had a similar effect to papaverine on ileum tissue when the tissue is stimulated with $BaCl_2$, and that carbachol had an antagonistic relationship with chelidonine. Chelidonine was also reported to be a musculotropic agent with a spasmolytic effect similar to papaverine [13], with possible antioxidant properties as a spasmolytic [12], as well as a

relative antagonistic relationship with acetylcholine [10]. This study has found that chelidonine responses showed a decrease compared to acetylcholine responses, and that acetylcholine applied together with chelidonine resulted in a comparatively smaller % increase in responses *(Fig. 2)*. Chelidonine is suggested to have an antagonistic effect with acetylcholine, which is consistent with the findings of previous studies. In a study that explored the effect of raw *C. majus* and *Corydalis lutea* extracts on isolated rat ileum tissues induced with acetylcholine, the contraction produced by acetylcholine was found to be antagonistic (12.7%) with *C. majus* extract applied in a dose of $2.0x10^{-4}$ g/mL. As a result, the study revealed that *C. majus* has an antispasmodic effect [10] On the other hand, studies show that the urinary bladder smooth muscle of various species (including humans) contains all muscarinic receptor subtypes, but the M2 and M3 receptors are dominant. However, it is emphasized that muscarinic M3 receptors are primarily responsible for contraction of the bladder smooth muscle [23,24]. While atropine has the same antagonistic effect on all subtypes of muscarinic receptors [24], oxybutinine has an antagonistic effect on the M3 receptor subtype with a higher affinity than other muscarinic subtypes [25]. Verapamil is a kind of calcium channel blocker [26]. In this study, it was observed that the relaxing effect of chelidonin in the urinary bladder was strengthened in the presence of atropine, verapamil and oxybutin ($P<0.01$) *(Fig. 3)*. In addition, the M2 and M3 subtypes of muscarinic receptors mediate effects in airway smooth muscles, and the M3 receptor plays an important role in contraction of airway smooth muscles [24]. At the same time, acetylcholine, which is synthesized in non-neuronal cells such as epithelial cells of the airways, also plays a role in the regulation of basic cell functions [27]. Carbachol, a cholinergic agonist substance, is more associated with the activation of muscarinic receptors (M3) [28], while mecamilamine is a potent ganglion blocker [29]. In this study, the contraction in tracheal tissue in response to cumulative doses of chelidonine (10^{-7}-10^{-3} M) is observed to show significant changes in the presence of carbacol *(Fig. 4)*, leading to the conclusion that chelidonine and carbachol have an antagonistic relationship, which is consistent with previous studies. This study also suggests that chelidonine is attracted to cholinergic muscarinic receptors; however, it reduces the effect of agonists due to its lower potency compared to acetylcholine. In addition, application of 1mM $CaCl_2$ to bladder tissue in non-calcium krebs solution after chelidonine and verapamil incubation was found to enhance chelidonine response *(Fig. 5)*, revealing the important role of the calcium ion in acetylcholine responses.

As with many potent natural products or mainly secondary plant metabolites, the active ingredient of greater celandine, chelidonine has been subject to several studies in the context of treatment of various cancer types. The cytotoxic effect mechanisms of greater celandine alkaloids show considerable variety, particularly in terms of their potential contribution to various cell death signal pathways. Previous studies showed remarkable differences in the DNA binding and sub-contribution capacity of benzophenanthridines isolated from this plant, with alkaloids such as sanguinarine and chelerythrine displaying high DNA binding capacity, while that of chelidonine was negligible [6,30]. On the other hand, it is associated with oxidative stress in lipid peroxidation, which results in the production of reactive oxygen species (ROS), affects the cell membrane and induces tissue damage, cell membrane deterioration and disruption. SOD and CAT are regarded as sensitive bio-indicators of oxidative stress, and are known to protect against ROS [31]. This study has shown that application of chelidonine in a concentration of 4 µg/mL to primary lung cells ($P<0.05$) and in concentrations of 2 and 4 µg/mL to primary kidney cells ($P<0.05$) resulted in statistically significant increase in CAT levels compared to the control group *(Fig. 7);* likewise, concentrations of 4 µg/mL in primary lung cells ($P<0.05$) and 1, 2 and 4 µg/mL in primary kidney cells ($P<0.05$, $P<0.01$, $P<0.05$) increased SOD levels significantly compared to control *(Fig. 8)*.

A study by Kaminskyy et al.[32] assessed the apoptotic, cytotoxic and DNA-damaging effects of isoquinoline alkaloids sanguinarine, chelerythrine and chelidonine on primary rat spleen cells and rat lymphocytic leukemia cell lines (L1210). According to the results of the study, chelidonine does not exhibit a clear cytotoxic or DNA-damaging effect on either cell types; however, it was observed to completely block cell growth in L1210 cells, preventing L1210 proliferation. In another study, chelidonine was assessed for its potential cytotoxic effect on pancreas, colon and breast cancer cell lines, primary endometrial cancer cells and murine pancreatic adenocarcinoma cells [5]. This study revealed that an application of 4 µg/mL of chelidonine to primary lung and kidney cells increased cell viability to its maximum (95.27% and 98.88%, respectively) *(Fig. 6)*. However, further increases in concentration reduced cell viability and increased cytotoxic effect.

In this study, it is found that chelidonine, the active ingredient in greater celandine, has a relaxant effect on urinary bladder and trachea tissues and that it is attracted to the cholinergic muscarinic receptors; however, it is also observed that the agent reduces the effect of agonists due to its lower potency compared to acetylcholine, and that calcium ions also play an important role in acetylcholine responses. As suggested before, chelidonine is a musculotropic agent with a spasmolytic effect similar to papaverine that acts through multiple mechanisms, and it may be mentioned that chelidonine acts through muscarinic receptors. The results of this study revealed that chelidonine has an effect on cholinergic receptors in the presence of agonists and antagonists. In addition, chelidonine caused a statistically insignificant increase in cell viability in primary lung and kidney cell lines at increasing doses (1 and 4 µg/mL), but this increase remained at the

control group level (P>0.05) *(Fig. 6)*. In contrast, chelidonine caused a significant decrease in cell viability at the same cell lines at doses of 8 µg/mL (respectively; P<0.01, P<0.05) and 16 µg/mL (P<0.01). In conclusion, it is suggested that the medicinal plant greater celandine and its active ingredient chelidonine might have beneficial effects on asthma, urinary incontinence and other urinary tract and respiratory diseases among others.

Conflict of Interest

The authors declared that there is no conflict of interest.

Statement of Author Contributions

E. Arslanbaş conceived the ideas of the study and writing manuscript; H. O. Doğan, H. Güngör, M. O. Atasoy and A. S. Kumru performed data collection and laboratory analysis; E. Arslanbaş, H. Kara and N. H. Turgut performed data analysis.

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