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ORIGINAL ARTICLE / ÖZGÜN MAKALE

VILDAGLIPTIN IMPROVES DETRUSOR CONTRACTILITY IN A MOUSE MODEL OF CYCLOPHOSPHAMIDE-INDUCED OVERACTIVE BLADDER

VİLDAGLİPTİN, SİKLOFOSFAMİD İLE İNDÜKLENEN AŞIRI AKTİF MESANE FARE MODELİNDE DETRÜSÖR KONTRAKTİLİTESİNİ İYİLEŞTİRİR

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ABSTRACT

Objective: *Overactive bladder (OAB) is a common urological disorder associated with detrusor overactivity linked to local tissue inflammation resulting in bladder hypersensitivity. The present study was aimed to investigate the therapeutic potential of vildagliptin (VIL), an anti-diabetic drug with anti-inflammatory effects, in a mouse model of cyclophosphamide (CP)-induced OAB.*
Material and Method: *To induce an animal model of OAB, female Balb/c mice were intraperitoneally (i.p) injected with CP (80 mg/kg) every two days for 7 days. Then, mice were orally treated with saline (OAB model), VIL (10 or 50 mg/kg/day) or solifenacin (10 mg/kg/day) for 7 consecutive days. On the 17th day of experiment, organ-bath experiments were performed using isolated mouse detrusor muscle to evaluate tissue contractility. In another set of mice, bladder inflammation was assessed by Evans blue extravasation.*
Result and Discussion: *Carbachol-induced contraction of detrusor strips significantly increased in OAB mice, which was reversed by treatment with VIL at 50 mg/kg or solifenacin. In addition, VIL treatment (50 mg/kg) reduced relative bladder weight and Evans blue dye extravasation into the bladders in CP-injected mice, demonstrating the inhibitory effect of VIL on CP-induced bladder inflammation. Our results showed that VIL ameliorated detrusor overactivity in a mouse model of CP-induced OAB by partially suppressing bladder inflammation.*
Keywords: *Bladder inflammation, detrusor, evans blue, overactive bladder, solifenacin, vildagliptin*

ÖZ

Amaç: *Aşırı aktif mesane (AAM), mesane aşırı duyarlılığına neden olan lokal doku inflamasyonuna bağlı detrüsör aşırı aktivitesi ile ilişkili yaygın bir ürolojik bozukluktur. Bu çalışma, siklofosfamid SFD) ile indüklenen AAM fare modelinde, anti-inflamatuar etkili anti-diyabetik bir ilaç olan vildagliptinin (VIL) terapötik potansiyelini araştırmayı amaçlamıştır.*
Gereç ve Yöntem: *AAM modelini indüklemek için dişi Balb/c farelere intraperitoneal olarak (i.p) 7 gün boyunca her iki günde bir SFD (80 mg/kg) enjekte edildi. Daha sonra fareler, art arda 7 gün boyunca oral olarak serum fizyolojik (AAM modeli), VIL (10 veya 50 mg/kg/gün) veya solifenasin (10 mg/kg/gün) ile tedavi edildi. Deneyin 17. gününde, mesane kontraktilitesini değerlendirmek için*

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izole fare detrüsör kası kullanılarak organ banyosu deneyleri yapıldı. Başka bir fare grubunda mesane inflamasyonu, Evans mavisi ekstravazasyonu ile değerlendirildi.
Sonuç ve Tartışma: *Karbakol ile indüklenen detrüsör kasılması, AAM modeli farelerinde önemli ölçüde arttı ve bu artış, VIL (50 mg/kg) veya solifenasin tedavisi ile düzeldi. Ayrıca; VIL tedavisi (50 mg/kg), SFD enjekte edilmiş farelerde rölatif mesane ağırlığını ve Evans mavi boyasının mesanelere ekstravazasyonunu azalttı. Bu sonuç, VIL'in SFD kaynaklı mesane inflamasyonu üzerindeki inhibitör etkisini ortaya koydu. Sonuçlarımız, VIL'in mesane inflamasyonunu kısmen baskılayarak SFD kaynaklı bir AAM fare modelinde detrüsör aşırı aktivitesini iyileştirdiğini ortaya koydu.*
Anahtar Kelimeler: *Aşırı aktif mesane, detrusor, evans mavisi, mesane inflamasyonu, solifenasin, vildagliptin*

INTRODUCTION

Overactive bladder (OAB) is a prevalent debilitating condition accompanied by frequency and nocturia, with or without urgency urinary incontinence and these lower urinary tract symptoms are often associated with detrusor overactivity defined as involuntary contractions of the detrusor smooth muscle [1]. Current pharmacotherapies of OAB include anti-muscarinic drugs and β3 adrenergic receptor agonists with limited efficacy and serious side effects leading to the poor compliance, which has led to high demand for novel drugs with improved efficacy and safety [2]. OAB is considered to have multifactorial etiology and its pathophysiology has not yet been completely elucidated. Recently, an inflammatory process has been reported to be involved in OAB and anti-inflammatory agents have been suggested to possess therapeutic potential for OAB [1,3].

Cyclophosphamide (CP) is one of most commonly used agent to induce an experimental animal model of OAB. It has been reported that multiple injection of CP caused OAB syndrome symptoms in mice [4-6]. The urotoxic metabolite acrolein is formed by hepatic microsomal enzymatic hydroxylation of CP and accumulates in the bladder, leading to urothelial damage and impaired urothelial barrier function by inducing bladder inflammation [7].

Dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin (VIL), sitagliptin, saxagliptin, linagliptin and alogliptin are orally effective drugs that improve glycemia in type 2 diabetes by preventing the degradation and thus increasing the level of incretins. Accumulating evidence suggests that they also exert pleiotropic effects beyond the glucose-lowering action [8]. Among DPP-4 inhibitors, VIL has recently gained attention due to its anti-inflammatory effects, which highlights the potential clinical repurposing of VIL for inflammation-related diseases [9-12]. The precise mechanisms of the anti-inflammatory effects of VIL remain elusive, however, toll-like receptors (TLRs) and nuclear factor kappa-B (NFκB) signaling pathways have been reported to be involved in the anti-inflammatory action of VIL [10-12].

In the present study, it was aimed to investigate the therapeutic effect of VIL in a mouse model of CP-induced OAB.

MATERIAL AND METHOD

Animals

Balb/c female mice weighing 25-35 g, aged 8-10 weeks, were used in this study. The mice were housed in cages at a constant temperature of 22 ± 2ºC under a 12 h light/12 h dark cycle, with free access to food and water. The experimental procedures were approved by Institutional Animal Care and Use Ethics Committee (approval number: 2023/45).

Chemicals and Drugs

VIL and solifenacin succinate were obtained from Ali Raif Pharmaceuticals and İlko Pharmaceuticals, Türkiye, respectively. Carbachol (CCh) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and CP (Endoxan®) was obtained from Baxter Oncology GmbH (Frankfurt, Germany). VIL, solifenacin and CP were freshly dissolved in 0.9% sterile saline prior to administration.

Induction of Experimental OAB Model and Treatments

Female Balb/c mice were randomly divided into six groups (n=11-14/group): *control*- saline-injected plus treatment with saline, *OAB group*- CP-injected plus treatment with saline, *OAB+VIL10*- CP-injected plus treatment with VIL at 10 mg/kg, *OAB+VIL50*- CP-injected plus treatment with VIL at 50 mg/kg, *OAB+SOL*- CP-injected plus treatment with anti-muscarinic drug, solifenacin at 10 mg/kg and *VIL50*- saline-injected plus treatment with VIL at 50 mg/kg. OAB was induced by totally four i.p injections of CP, as described previously [6]. CP (80 mg/kg, i.p) was administered every other day for 7 consecutive days. Control and VIL50 groups received only saline injections. 2 days after the fourth CP and saline injection, mice were treated with VIL (10 and 50 mg/kg/day), solifenacin succinate (10 mg/kg/day) or saline for 7 consecutive days. On the 17th day of the experimental protocol, the mice were euthanized by cervical dislocation and the whole urinary bladders were harvested for contractility studies or Evans blue extravasation assay (Figure 1). Mice were given saline, VIL and solifenacin orally via a gavage needle. The experimental protocol and doses of the drugs were chosen based on previous studies [5,9,13]. Relative bladder weights of all groups were determined by calculating the ratio of wet bladder weight (mg) to body weight (g) of mice to evaluate bladder hypertrophy as a marker of inflammatory edema [14,15].

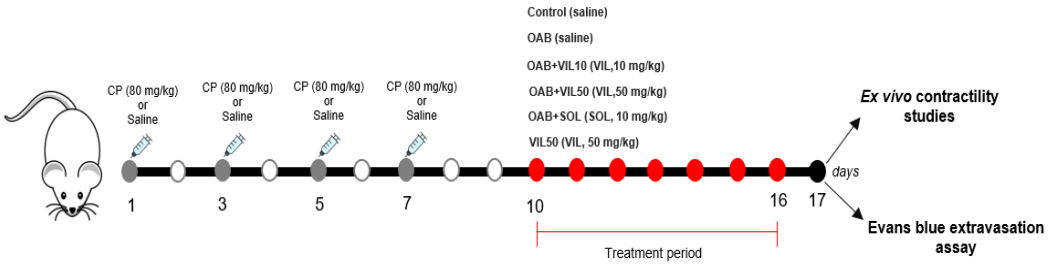

Figure 1. Schematic representation of the experimental design. Mice were treated once a day through the treatment period. VIL and solifenacin were given orally. CP, cyclophosphamide; OAB, overactive bladder; SOL, solifenacin; VIL, vildagliptin.

Contractility Studies

In a set of mice in each group (n=6-8/group), one detrusor smooth muscle strip was prepared from each urinary bladder, as described previously [16]. Detrusor strips were suspended longitudinally between an isometric force transducer (MAY FDT-10A Force Displacement Transducer, Commat, Ankara, Türkiye) and a steel curved hook within a 30 ml water-jacketed tissue baths containing Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM NaH_2PO_4, 1.3 m M $MgSO_4$ 1.3, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose) continuously bubbled with 95% O_2 and 5% CO_2 at 37°C. Changes in isometric force were recorded using a MP35 data acquisition system (Biopac Systems, Goleta, CA, USA). Each strip was stretched to 1 g of tension and allowed to equilibrate for 1 hour while changing bath solution every 20 min. Then, strips were firstly challenged with KCl (80 mM) to test tissue viability. After an equilibration period, CCh (10^{-8}-10^{-4} M) was added to bathing solution to obtain cumulative concentration-response curves. CCh-induced contractile responses of strips were normalized to the weight of each strip and expressed as milligram tension per milligram strip weight [16,17].

Evans Blue Extravasation Assay

In another set of mice (n=5-6/group), Evans blue extravasation into the bladders were measued to assess bladder inflammation. Briefly, Evans blue dye (0.5% w/v in saline, 200µl) was injected intravenously via tail vein 30 min before euthanasia. The bladders were dissected from the body, weighed, sliced longitudinally, and incubated in 1 ml formamide at 56°C for 24 hours for dye extraction. Then, the samples were centrifuged at 14.000 g for 45 minutes at 4°C and the supernatants were

collected. The concentration of extracted dye was determined by measuring the absorbance with a spectrophotometer at 620 nm, followed by a standard curve. The results were expressed as in μg of Evans blue/g of tissue [18,19].

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). GraphPad Prism software (Graphpad Prism 5.0.1, San Diego, CA, USA) was used for statistical analyses and graphical presentation. Comparisons of groups were carried out through analysis of variance (ANOVA) followed by Bonferroni test for contractility studies or student's *t*-test for Evans blue extravasation assay and relative bladder weights. For CCh-induced concentration-response curves, the values of E_{max} (the maximum response) and the negative logarithm of the concentration to produce the half of maximal response (pEC_{50}) were calculated using nonlinear regression plots (GraphPad Prism version 5.0.1, GraphPad, San Diego, CA, USA).

RESULT AND DISCUSSION

VIL Attenuated the CCh-induced Contractions of Detrusor Strips in CP-induced OAB

CCh (10^{-8}-10^{-4} M) produced concentration-dependent contractions in the strips of all groups (Figure 2a). In OAB group, the maximal contractile responses of detrusor strips to CCh (10^{-8}-10^{-4} M) were significantly increased compared to control group mice ($p<0.001$; E_{max}=747.00 ± 43.43 and 456.50 ± 39.45 mg tension/mg tissue, respectively), indicating that multiple injections of CP led to OAB in mice. Treatment with VIL at 50 mg/kg for 7 days caused a significant decrease in CCh-induced contractions in the detrusor strips of CP-injected mice compared to OAB group ($p<0.05$; E_{max}=566.80 ± 36.98 and 747.00 ± 43.43 mg tension/mg tissue, respectively). Solifenacin, an approved drug for OAB, markedly decreased the maximum contractile response to CCh in CP-injected mice compared with OAB group ($p<0.01$; E_{max}= 547.10 ± 55.27 mg and 747.00 ± 43.43 mg tension/mg tissue, respectively). However, VIL treatment at 10 mg/kg failed to improve detrusor overactivity in CP-injected mice. In VIL50 group, CCh-induced contractile response was similar with control group ($p>0.05$). No difference was observed in pEC_{50} values of all groups for CCh-induced contractions (Figure 2b and Table 1).

Table 1. E_{max} and pEC_{50} values for CCh-induced contractions of the detrusor strips from all groups

Groups	E_{max} (mg tension/mg tissue)	pEC_{50}
Control	456.50 ± 39.45	5.79 ± 0.16
OAB	747.00 ± 43.43**	5.84 ± 0.17
OAB + VIL10	685.00 ± 66.42*	5.73 ± 0.23
OAB + VIL50	566.80 ± 36.98$^{\#}$	5.55 ± 0.11
OAB + SOL	547.10 ± 55.27$^{\#}$	5.97 ± 0.19
VIL50	495.90 ± 65.07$^{\#\#}$	5.55 ± 0.21

Data were expressed as mean ± SEM (n = 6-8). **p<0.01, *p<0.05 compared with control group. $^{\#\#}$p<0.01, $^{\#}$p<0.05 compared with OAB group. CCh, carbachol; E_{max}, maximal contraction evoked by carbachol.OAB, overactive bladder; pD2, the negative logarithm of the EC50; SOL, solifenacin; VIL, vildagliptin

VIL Suppressed CP-induced Bladder Inflammation

In OAB group, CP injection significantly ($p<0.01$) increased evans blue extravasation in the bladders compared to control group (48.25 ± 4.28 and 18.28 ± 6.92 μg/g of tissue, respectively), which was markedly ($p<0.05$) blunted by VIL treatment at 50 mg/kg (30.24 ± 3.57 μg/g of tissue). However, treatment with solifenacin (35.20 ± 6.35 μg/g of tissue) or VIL at 10 mg/kg (42.00 ± 9.47 μg/g of tissue) did not alter evans blue extravasation stimulated by CP (Figure 3a). In addition, we determined relative bladder weights of all groups for the assessment of bladder hypertrophy as a hallmark of bladder inflammation. The relative bladder weight dramatically increased in OAB group (0.83 ± 0.03) compared to control group (0.60 ± 0.02, $p<0.001$). VIL treatment at 50 mg/kg caused a significant ($p<0.05$) decrease in relative bladder weight compared to OAB group (0.71 ± 0.01 and 0.83 ± 0.03, respectively).

Solifenacin (0.83 ± 0.02) or VIL at 10 mg/kg (0.86 ± 0.04) did not reduce the relative bladder weight of the mice with OAB (Figure 3b). The body weights of mice were similar among groups.

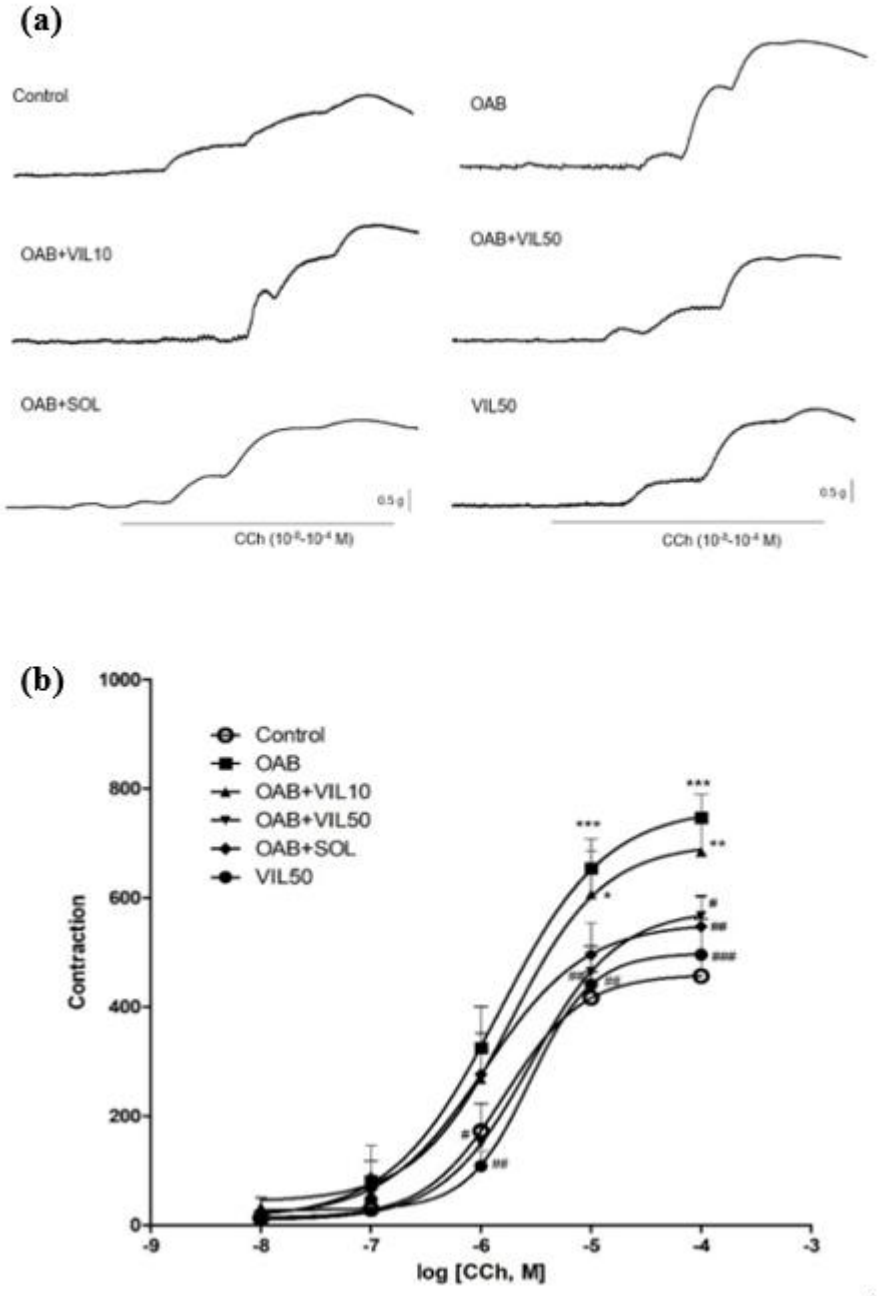

Figure 2. VIL improved detrusor overactivity in mice with OAB induced by CP. (a) Representative original traces of CCh-induced contractions of the detrusor strips in all groups. (b) Cumulative concentration-response curves for CCh (10^{-8}-10^{-4} M). Data were expressed as mean ± SEM (n=6-8). ***p<0.001, **p<0.01, *p<0.05 compared with control group. ###p<0.001, ##p<0.01, #p<0.05 compared with OAB group. CCh, carbachol; OAB, overactive bladder; SOL, solifenacin; VIL, vildagliptin

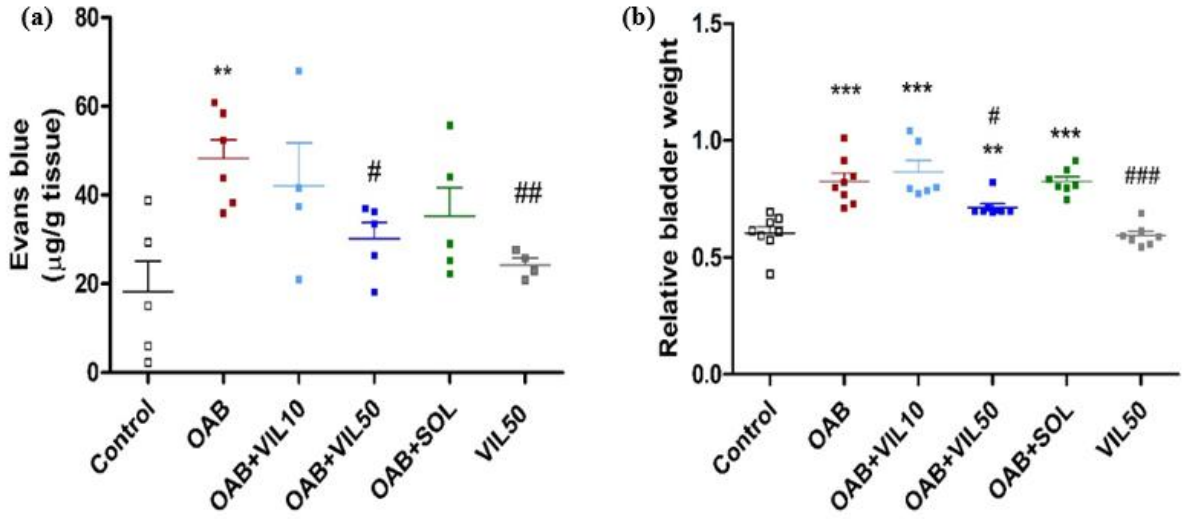

Figure 3. VIL attenuated bladder inflammation in mice with OAB induced by CP. (a) Quantitative analysis of Evans blue leakage. (b) The relative bladder weights of all groups. Data were expressed as mean ± SEM (n=4-8). ***p<0.01,**p<0.01 compared with control group. $^{###}$p<0.001, $^{##}$p<0.01, $^{#}$p<0.05 compared with OAB group. OAB, overactive bladder; SOL, solifenacin; VIL, vildagliptin

OAB is a urological syndrome typically manifested by bothersome symptoms of urgency, with or without urge incontinence, and often accompanied with increased urinary frequency and nocturia, resulting in impaired social functioning and quality of life. It is a common urological disease with an overall prevelance of around 17%, mostly affecting elderly people [1,20]. OAB is considered to be caused by multiple factors related with functional alterations in bladder, mainly leading to involuntary detrusor contractions defined as detrusor overactivity. However, the precise etiology of OAB has not largely understood [21]. Currently avaliable pharmacological agents for OAB including anti-muscarinics and β3 agonists have repoted to be associated with several side effects and limited efficacy. Therefore, physiopathological mechanisms of OAB has been under intense investigation to discover novel therapeutic targets, and thus to develop new drugs [22]. Bladder inflammation has gained a remarkable interest, which has a direct effect on bladder contractility [23]. Several lines of evidence suggests that various inflammatory process or cytokines are involved in the pathogenesis of OAB [2]. Until now, experimental studies have reported that OAB is linked to bladder inflammation and anti-inflammatory agents have efficacy in OAB models [24,25]. Moreover, elevated levels of urine cytokines and the signs of inflammation in bladder specimens have been detected in the patients with OAB [26]. Therefore, anti-inflammatory-based approaches may offer hope for the treatment of OAB [27].

DPP-4 inhibitors including sitagliptin, vildagliptin (VIL), saxagliptin, alogliptin, linagliptin, dutogliptin etc. are a class of drugs widely used for the treatment of type 2 diabetes. They are highly effective in glucose-lowering by inhibiting the enzymatic degradation of incretins, thus amplifying the effects of incretins. Due to their favorable safety and efficacy profile, they have become one of the most preferred anti-diabetic drugs [28,29]. In recent years, the anti-inflammatory potential of DPP-4 inhibitors have attracted more attention beyond glycemic control [30,31]. VIL, a well-known DPP-4 inhibitor, has been also reported to suppress inflammation in various *in vivo* and *in vitro* models [9-12]. Liu and Qia showed that VIL treatment (10 mg/kg for 21 days) significantly decreased the expression of pro-inflammatory cytokines and the infiltration of inflammatory cells in lung tissues of bleomycin-treated mice [9]. Also, in a rat model of cyclosporine A-induced hepatotoxicity, VIL treatment (10 mg/kg for 28 days) improved liver inflammation by suppressing the nuclear factor-kappa B (NF-κB) activity [10]. In another study conducted by Sherif et al., VIL was reported to exhibit hepatoprotective effect by reducing inflammation through the downregulation of TLR4/NF-κB signaling pathway in a rat model of hepatic ischemia/reperfusion injury [11]. Moreover, treatment with VIL (5 or 10 mg/kg for 21 days) attenuated colonic inflammation by inhibiting PI3K/Akt/NFκB pathway in rats with colitis

induced by acetic acid [12]. However, the effect of VIL on bladder inflammation is completely unknown.

CP-induced bladder inflammation is a widely used and well-established experimental model for OAB. Several studies have reported that multiple injections of CP cause voiding dysfunction similar to the symptoms of OAB syndrome in mice [4-6]. Acrolein, the urotoxic metabolite of CP, leads to urothelial injury resulting in inflammation and bladder dysfunction [4,32].

In the present study, we investigated the effect VIL on CP-induced OAB. To test the effect of VIL on detrusor contractility, we evaluated *ex vivo* contraction of the strips to CCh by using isolated tissue bath. In addition, we used Evans blue extravasation assay to test the effect of VIL on bladder inflammation induced by CP. To our results, four injections of CP significantly increased the CCh-induced contraction of detrusor strips in line with previous studies [33,34]. The increase in CCh-induced contraction was blunted by solifenacin, a potent and long acting anti-muscarinic drug. We also found that high dose of VIL (50 mg/kg) caused a marked decrease in CCh-induced contractile response of the strips in CP-treated mice. Furthermore, we found that CP significantly increased evans blue extravasation into bladder tissue and relative bladder weight, indicating that CP caused inflammation in bladder tissue, which is similar to previous studies [14,35,36]. Solifenacin did not ameliorate bladder inflammation although it was effective in attenuating detrusor overactivity in CP-treated mice, demonstrating that solifenacin offers symptomatic treatment in CP-induced OAB model. However, high dose of VIL (50 mg/kg) considerably reduced extravasation and relative bladder weight as well as the CCh-induced contractions of the strips in CP-treated mice. This finding suggests that VIL could interfere with bladder inflammation underlying detrusor overactivity in CP-induced OAB model. All together, our results demonstrated that treatment with VIL (50 mg/kg/day for 7 days) effectively improved detrusor contractility by partially suppressing bladder inflammation in a mouse model of CP-induced OAB.

Despite providing evidence for the effect of VIL on CP-induced OAB, our study has certain limitations. In the present study, the molecular mechanisms of VIL mediating its anti-inflammatory effect has not been identified. Further studies are needed to test the role of signaling pathways such as PI3K/Akt/NFκB, TLR4/NF-κB etc. in the therapeutic effect of VIL. In addition, the level of the expression of pro-inflammatory cytokines can be directly measured in the bladder tissues to support the reported anti-inflammatory effect of VIL.

Overall, the present study provides the first evidence that VIL has beneficial effect in CP-induced OAB by suppressing bladder inflammation. Our results suggest that VIL might be repurposed as a promising therapeutic candidate for the treatment of OAB. Therefore, further molecular and clinical studies are needed to identify the mechanism of these effects and confirm current preclinical results in the future research.

AUTHOR CONTRIBUTIONS

Concept: S.E.; Design: S.E.; Control: S.E., E.N.B.; Sources: S.E., E.N.B.; Materials: S.E., E.N.B.; Data Collection and/or Processing: S.E., E.N.B., M.I.K., M.N.Y.; Analysis and/or Interpretation: S.E., E.N.B.; Literature Review: S.E., M.I.K., M.N.Y.; Manuscript Writing: S.E.; Critical Review: S.E., E.N.B., M.I.K., M.N.Y.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

All experimental procedure of the animals was approved by Animal Experiments Local Ethics Committee of Karadeniz Technical University (Approval no: 2023/45).

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