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Protective effect of high water-soluble curcuminoids on voiding dysfunction and urinary bladder hypercontractility in cyclophosphamide-induced overactive bladder in mice

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ABSTRACT: Overactive bladder (OAB) is characterized by urinary frequency, urgency and incontinence. Curcuminoids, the active compounds found in *Curcuma longa* Linn., exhibit anti-inflammatory, antioxidant, and antimicrobial properties. However, their clinical application is limited due to poor water solubility and low absorption. This study aimed to investigate the effects of highly water-soluble curcuminoids (HWC) formulated using a ternary inclusion complex system on voiding patterns and bladder contractile properties in OAB-induced mice. HWC were prepared using a green extraction process and a ternary inclusion complex system composed of curcuminoids, hydroxypropyl-β-cyclodextrin, and polyvinylpyrrolidone K30. Adult male mice were orally administered with HWC (50 mg/kg BW) or vehicle once daily for seven days. OAB was induced by an injection of cyclophosphamide (CYP, 150 mg/kg, i.p). CYP-treated mice showed a significant increase in the total number of urine spots, urine area, and the percentage of small urine spots. Bladders from CYP-treated group showed increased tone, amplitude, and frequency of contraction in response to carbachol, a muscarinic agonist, which was reversed with HWC administration. In summary, administration of HWC was associated with attenuation of CYP-induced alterations in voiding pattern and bladder hypercontraction through modulation of muscarinic signaling in the urinary bladder.

KEYWORDS: bladder contractility, cyclophosphamide, high water-soluble curcuminoids, overactive bladder, voiding spot analysis

INTRODUCTION

Overactive bladder (OAB) symptoms, including urinary frequency, urgency, and incontinence, have been reported to significantly impair quality of life and social activities [1]. OAB affects approximately 16% of adults worldwide in both sexes, with prevalence increasing with age [2]. Although the etiology of OAB remains inconclusive, previous studies have suggested that hypersensitivity of bladder afferent nerves and dysregulation of the detrusor muscle activity are key contributing factors [3]. An established animal model for inducing OAB-like symptoms involves intraperitoneal injections of the chemotherapeutic agent cyclophosphamide (CYP). Following administration, CYP is metabolized to acrolein by hepatic enzymes; this metabolite is excreted in the urine and induces inflammation of the urinary tract [4]. A previous study demonstrated that a single intraperitoneal injection of CYP (150 mg/kg) induces OAB-like characteristics in mice [5]. Moreover, administration of CYP (200 mg/kg, i.p.) caused acute cystitis, while repeated injections (75 mg/kg, four times over seven days) produced chronic cystitis, characterized by bladder overactivity, marked structural alterations, and increased mast cell infiltration (mastocytosis) within the bladder [6]. Lui et al [7] further reported that daily CYP injections (80 mg/kg for seven consecutive days) induced cystitis in mice.

Currently, the main pharmacological treatments for OAB are antimuscarinic agents such as oxybutynin. However, these drugs often produce undesirable side effects, including dry mouth, constipation, and blurred vision [8]. Consequently, there is growing interest in identifying alternative natural compounds with therapeutic potential for OAB that offer comparable efficacy but fewer adverse effects. Curcuminoids are natural polyphenolic compounds derived from turmeric (*C. longa* L.), which consist of curcumin, demethoxycurcumin, and bis-demethoxycurcumin [9, 10]. Previous studies have shown that curcumin exhibits potent anti-inflammatory and antioxidant effects in several organs, including the intestine, stomach, and urinary bladder [9]. Interestingly, curcumin has also been reported to inhibit the contractility of the intestine and isolated urinary bladder tissues [11, 12] and to reverse carbachol-induced colonic contraction [13].

However, the therapeutic application of curcuminoids is limited by their poor water solubility. A previous investigation developed a green extraction method

to prepare highly water-soluble curcuminoids (HWC) using a ternary complex composed of curcuminoids, hydroxypropyl-β-cyclodextrin, and polyvinylpyrrolidone K30, which enhances water solubility up to 90% [14]. HWC prepared via this green extraction method has also shown anticancer activities [15, 16], reduced osteoclastogenic activity [17], and promoted osteoblast differentiation [18]. Nevertheless, it remains unclear whether HWC exerts a protective effect on bladder overactivity in CYP-induced OAB.

Therefore, this study aimed to investigate the effects of oral administration of HWC obtained through a green extraction method on voiding patterns and bladder contractility in CYP-induced bladder overactivity.

MATERIALS AND METHODS

Preparation of HWC

Curcuminoid extract (88% w/w) was prepared from turmeric (*C. longa* L.) powders using a green extraction and fractionation method as previously described [15]. Briefly, the dried *C. longa* powders were extracted with ethanol using a microwave-assisted extraction method (900 W) at 70 °C, consisting of three irradiation cycles (each cycle comprising 3 min power-on, and 30 s power-off) using a microwave oven (LG Electronics Inc., Bangkok, Thailand). The extract was filtered and then subjected to a Diaion® HP-20 column (Merck KGaA, Darmstadt, Germany), which was eluted sequentially with 55% and 60% v/v ethanol. A HWC formulation (containing 14% w/w curcuminoids) was subsequently prepared as a ternary complex using the solvent evaporation method [16]. First, a binary complex of curcuminoid extract and hydroxypropyl-β-cyclodextrin (Merck KGaA) was prepared at a 1:1 molar ratio. The ternary complex (HWC) was then obtained by dispersing the binary complex with polyvinylpyrrolidone K30 (9% w/w) (Merck KGaA). The resulting HWC demonstrated approximately 90% water solubility, as previously reported [16].

Animals and treatments

Adult male Institute of Cancer Research (ICR) mice (6–8 weeks old) were obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. All experimental procedures were approved by the Institutional Animal Care and Use Committee, Prince of Songkla University (protocol code: 2561-01-088). Animals were acclimatized for at least seven days under standard laboratory conditions with a 12 h light/dark cycle, a controlled temperature of 25 ± 2 °C, and free access to water and a standard diet (S.W.T., Thailand).

Mice were randomly assigned to one of three groups: (i) control group (n = 8), (ii) CYP group (n = 7), and (iii) HWC group (n = 8). Animals in the HWC group received oral administration of HWC (50 mg/kg body weight) once daily for seven consecutive days. On day 5, the animals received HWC by oral gavage one hour prior to CYP injection.

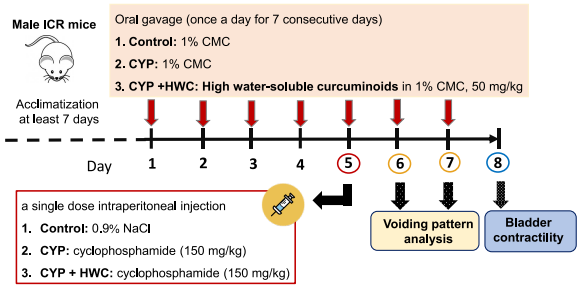

Fig. 1 Schematic diagram showing the experimental timeline of this study.

Mice in the control and CYP groups were orally administered 1% carboxymethyl cellulose (CMC) as a vehicle for HWC. The control group additionally received normal saline injection (i.p.) as the vehicle control. Voiding patterns were investigated on days 6 and 7 of treatment. On day 8, the animals were sacrificed, and the urinary bladder was collected, weighed, and subjected to contractility assessment. A schematic diagram illustrating the experimental timeline is depicted in Fig. 1.

Voiding spot analysis

To evaluate urinary bladder function, voiding patterns were assessed in all groups using voiding spot analysis on days 6 and 7 of the experimental protocol. Mice were placed in the standard cage lined with a wire mesh positioned above filter paper (Whatman™ Grade1, Cat No. 18023133). Each mouse was kept in the cage for 4 h, from 12:00 am to 4:00 pm, with free access to food but without access to water. After the collection period, the filter paper was visualized under UV light, and images were captured for analysis. The total number of urine spots, total urine area, and the percentages of small and large urine spots were quantified using Image J Software [19, 20].

In vitro organ bath technique

One day after completing the treatment, mice were anesthetized with thiopental sodium (70 mg/kg, i.p.), and abdominal cavity was opened. The urinary bladder was excised and trimmed into a rectangular strip, which was then mounted in an organ bath containing 20 ml oxygenated Krebs solution (composition in mM: NaCl 119, KCl 4.5, $MgSO_4$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, glucose 11.1). The solution was maintained at 37 °C and continuously aerated with a mixture of 95% O_2 and 5% CO_2. Tissues were allowed to equilibrate for 30 min under a resting tension of 1.5 g. Bladder contractility was recorded using a force transducer connected to a data acquisition system

(LabChart7, AD Instruments, Australia) to determine the tone, amplitude, and frequency of spontaneous contractions.

Statistical analysis

Data are expressed as the mean ± standard error of the mean (S.E.M.). Statistical analyses were carried out using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for comparisons between all experimental groups and the control group. Body weight and bladder contractile responses to cumulative concentrations of carbachol were analyzed using two-way repeated-measures ANOVA. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were conducted using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Changes in body weight and bladder weight

There was no significant difference in body weight among the groups (Fig. 2a). However, the bladder tissue weight in the CYP group (0.97 ± 0.07 mg/g) significantly increased compared with the control group (0.76 ± 0.039 mg/g). Interestingly, oral administration of HWC (50 mg/kg) markedly reduced bladder weight in the CYP + HWC group compared with the CYP group (Fig. 2b).

Oral administration of HWC reversed changes in voiding patterns in CYP-treated mice

Representative images of urine spots from each group are shown in Fig. 3a–c. Mice in the control group exhibited a regular voiding pattern characterized by a large urine spot typically located in a corner of the filter paper. In contrast, CYP-treated mice displayed sparse and small urine spots. Interestingly, CYP-treated mice receiving oral HWC showed attenuation of these alterations in voiding behavior. The number of urine spots significantly increased in the CYP group (30 ± 3.77) compared with the control group (13.43 ± 4.80), and this change was reversed in the CYP + HWC group (26.57 ± 4.56) (Fig. 3d). The total urine area, which reflects the overall urine volume, was markedly increased in CYP-induced mice (229.7 ± 44.15 cm^2) compared with the controls (110.7 ± 22.97 cm^2). Administration of HWC significantly reduced the total urine area in CYP-treated mice (185.3 ± 37.30 cm^2) (Fig. 3e). Moreover, the percentage of small urine spots (< 0.3 cm^2) was significantly higher in the CYP group (14.96 ± 2.23%) than in the control group (4.57 ± 2.82%) (Fig. 3f). Conversely, the proportion of large urine spots (> 30 cm^2) was significantly decreased in the CYP-treated group (3.96 ± 1.44%) compared with the control group (31.50 ± 7.81%), and this reduction was reversed by oral administration of HWC (13.26 ± 8.28%) (Fig. 3g). Collectively, these results indicate that oral administration of HWC effectively reversed CYP-induced alterations in urinary voiding patterns associated with bladder overactivity.

Oral administration of HWC reversed responses to KCl-induced bladder hypercontractility in CYP-treated mice

Representative traces of basal bladder contractions from each group are shown in Fig. 4a. Bladder strips from CYP-treated mice exhibited a significantly increased tonic contraction in response to KCl (80 mM) compared with those from control mice (Control: 346.8 ± 41.64% baseline; CYP: 532.30 ± 63.23% baseline). This heightened contractile response was markedly attenuated in the CYP + HWC group (408.7 ± 29.68% baseline, $p < 0.05$) (Fig. 4b). Similarly, the frequency of bladder contraction in response to KCl was significantly elevated in the CYP group (Control: 53.71 ± 14.13% baseline; CYP: 148.40 ± 32.38 % baseline), whereas HWC administration significantly reduced this increase (63.21 ± 13.97% baseline, $p < 0.05$) (Fig. 4c). In contrast, no significant differences were observed in the amplitude of bladder contractions among the groups (Control: 69.67 ± 22.46% baseline; CYP: 106.00 ± 22.98% baseline; CYP + HWC: 90.48 ± 11.70 % baseline). Collectively, these findings indicate that CYP-treated mice exhibited KCl-induced bladder hypercontractility, which was effectively ameliorated by oral administration of HWC.

Bladders of CYP-treated mice exhibited enhanced responses to carbachol (CCh), which were reversed by HWC treatment

Bladder contractile response to cumulative concentrations of CCh (0.1, 1, 10, and 100 μM) was examined in isolated bladder tissues from all groups. Representative traces of bladder contractions are shown in Fig. 5a. CYP-treated mice displayed significantly increased tonic contractions in response to CCh at 1, 10, and 100 μM compared with the control group. In contrast, tonic contractions in the CYP + HWC group did not differ significantly from those of the control group ($p < 0.05$) (Fig. 5b). Similarly, the frequency of bladder contractions was significantly higher in CYP-treated mice in response to CCh at 1 μM than in the control groups. This increase was normalized in the CYP + HWC group ($p < 0.05$) (Fig. 5c). In addition, the amplitude of contractions in response to CCh at 100 μM was markedly elevated in the CYP group compared with controls, and this effect was significantly attenuated by HWC treatment ($p < 0.05$) (Fig. 5d). Collectively, these findings suggest that oral administration of HWC effectively reversed the exaggerated bladder contractile responses to muscarinic stimulation observed in CYP-treated mice. A schematic summary of the experimental findings is presented in Fig. 6.

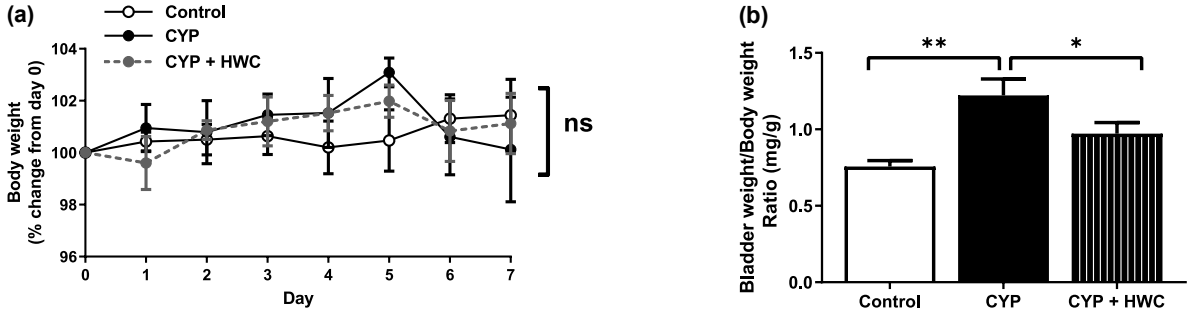

Fig. 2 Oral administration of high water-soluble curcuminoids reversed an increase in bladder weight of cyclophosphamide-treated mice. (a) Changes in body weight of animals in all groups (Repeated two-way ANOVA, ns = not significant, $n = 6$–7). (b) Bladder weight of animals in all groups. (* $p < 0.05$, ** $p < 0.01$, ns = not significant, One-way ANOVA with Dunnett's multiple comparisons vs. control group, $n = 6$–7).

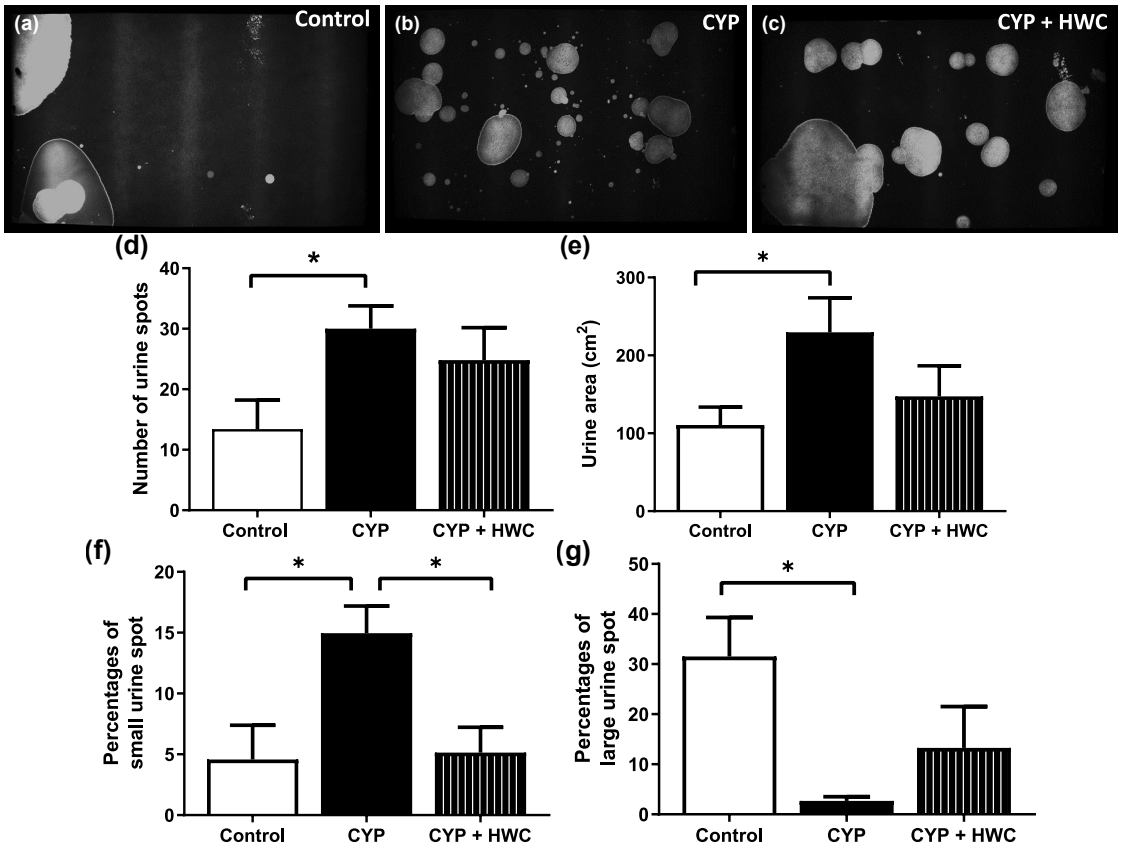

Fig. 3 Representative images of urine voiding patterns of (a) control group, (b) CYP group, (c) HWC administration group (CYP + HWC). One-way ANOVA with Dunnett's multiple comparisons vs. CYP group, * $p < 0.05$, $n = 6$–7).

DISCUSSION

This study investigated the therapeutic potential of HWC, a structurally modified curcuminoids, in preventing urinary bladder overactivity induced by CYP in mice. Oral administration of HWC effectively ameliorated voiding dysfunction. The bladder weight of CYP-treated mice was significantly higher than that of control mice, whereas this increase was reversed in mice receiving HWC treatment. These findings suggest that CYP induces inflammatory changes in the urinary bladder, which can be attenuated by treatment with HWC. Our observations are consistent with previous reports demonstrating that CYP induces overactive bladder symptoms accompanied by bladder inflammation, including edema, urothelial hyperplasia, and elevated urinary NGF levels [4, 20, 21].

We also observed abnormal voiding patterns in

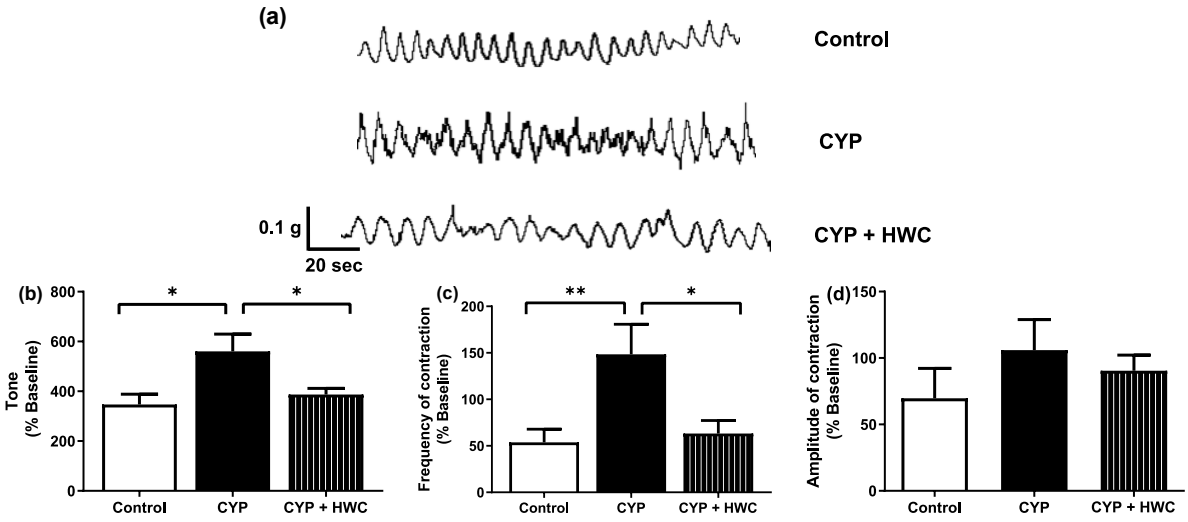

Fig. 4 CYP-treated mice increased tonic and frequency of bladder contraction in response to KCl, which was attenuated with HWC administration. (a) Sample traces represented spontaneous basal contraction of bladder tissues in all groups, (b) tonic contraction, (c) frequency of contraction, and (d) amplitude of contraction of the bladder strips in all groups (* $p < 0.05$, ** $p < 0.01$, One-way ANOVA with Dunnett's multiple comparisons vs. CYP group, $n = 6$–7).

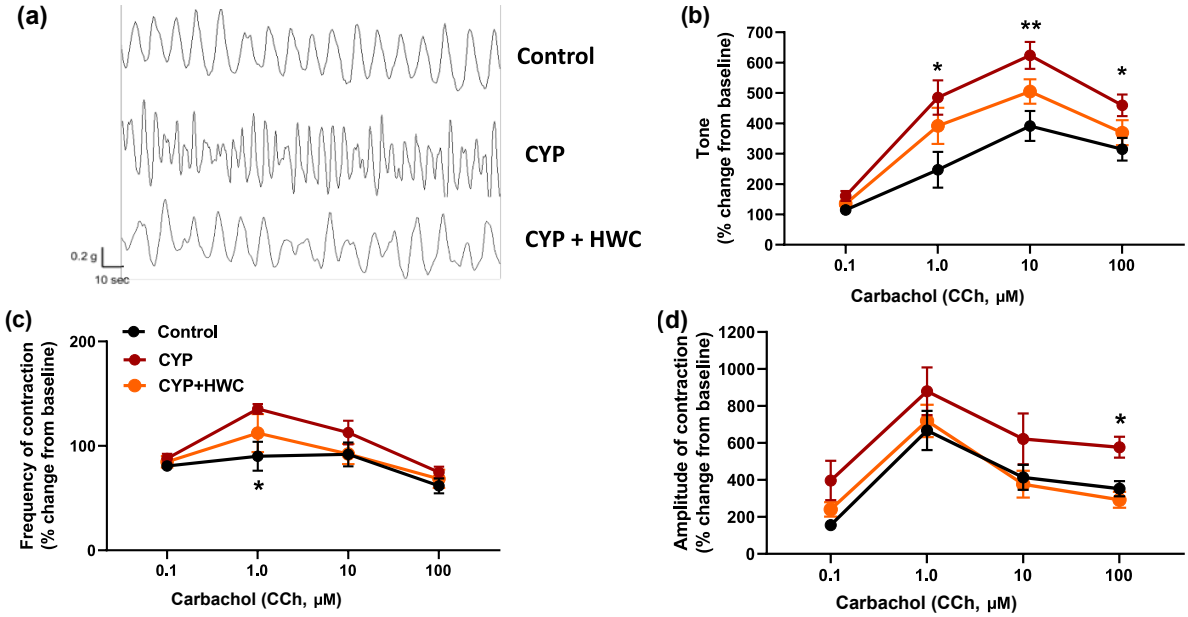

Fig. 5 Oral administration of HWC decreased contractile responses to muscarinic stimulation in CYP-treated mice. (a) Representative tracing of spontaneous contraction of bladder tissues in response to carbachol, (b) tonic contraction, (c) frequency of contraction, and (d) amplitude of contraction of bladder strips in response to cumulative concentrations of carbachol (CCh) (* $p < 0.05$, ** $p < 0.01$, repeated two-way ANOVA with Dunnett's multiple comparisons vs. control group, $n = 6$–7). In subfig. (b) to (d), X-axes, change the label to Carbachol (CCh, μM) and remove μM from all the numbers under the axes.

CYP-treated mice, indicative of overactive bladder-like symptoms, such as increased total urine area, increased number of urine spots, and a greater proportion of small urine spots, along with a decrease in large urine spots. These findings are consistent with previous studies that reported altered voiding behavior in CYP-treated rodents. Boudes et al [22] demonstrated that mice receiving CYP (40 and 80 mg/kg, every 2 days for 7 days) exhibited an increased number of urine spots with a reduced voided volume per micturition. Similarly, intraperitoneal administration of CYP (200 mg/kg) reduced micturition intervals in rats. Moreover, CYP-induced cystitis in rats has been associated with altered urodynamic parameters, in-

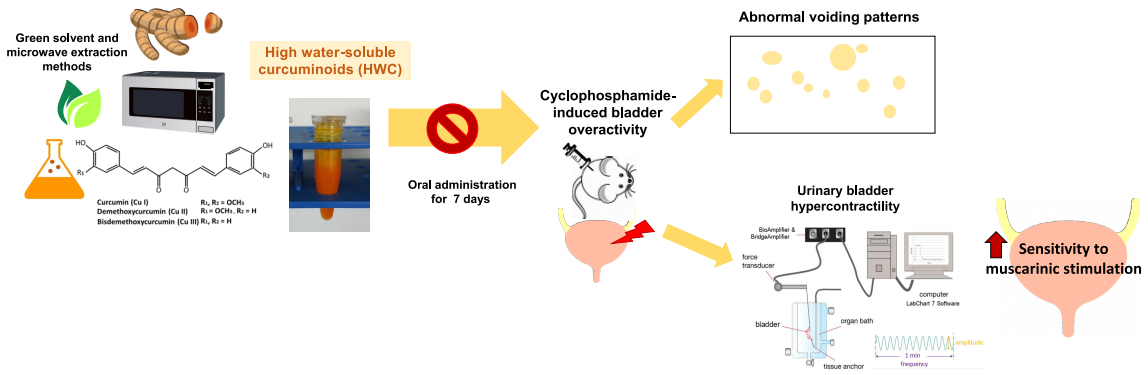

Fig. 6 A schematic diagram summarizing the effects of HWC administration on CYP-induced bladder overactivity.

cluding shortened voiding intervals, increased urinary frequency, and elevated voiding pressure, all of which are indicative of bladder hyperactivity [23].

In the present study, oral administration of HWC (50 mg/kg BW) for seven consecutive days prior to CYP-induced bladder overactivity effectively prevented abnormal voiding patterns in mice. This protective effect is in agreement with previous findings showing that curcumin (200 mg/kg i.p.) exerts a uroprotective effect in CYP-induced cystitis in rats [24]. Furthermore, the beneficial effects of orally administered curcumin have been demonstrated in other inflammatory models, such as gastric inflammation, where curcumin (20–80 mg/kg) dose-dependently reduced inflammatory responses through its antioxidant activity and inhibition of nitric oxide and pro-inflammatory cytokine production [25]. However, the observed effects in this study are interpreted based on the known anti-inflammatory and antioxidant activities of curcuminoids reported in previous investigations, while the direct evidence of tissue delivery and mechanistic confirmation in the urinary bladder was beyond the scope of the present study.

Interestingly, bladder contractile properties of CYP-treated mice exhibited heightened responses to both KCl and muscarinic receptor stimulation. In contrast, a previous study reported that repeated administration of CYP (75 mg/kg, every third day for 10 days) in rats decreased muscarinic and purinergic responsiveness in the urinary bladder [23]. This discrepancy in muscarinic response between studies may be attributed to differences in experimental design, such as species variation, drug dosage, and induction protocol. Andersson et al [26] further demonstrated that CYP-induced cystitis in rats alters cholinergic signaling in the urinary bladder through enhanced nitric oxide production mediated by M3/M5 muscarinic receptor activation. Consistently, inhibition of nitric oxide synthase was shown to prevent muscarinic and purinergic alterations in CYP-induced cystitis [26, 27].

In the present study, HWC administration reversed the enhanced contractile responses to both KCl and carbachol observed in CYP-treated mice. This finding aligns with a previous investigation in colonic tissues showing that curcumin reverses carbachol-induced colonic contraction [12]. Although the current study did not elucidate the precise mechanisms underlying the protective effects of HWC against CYP-induced bladder hypercontraction, previous evidence suggests that curcumin may exert direct inhibitory actions on detrusor muscle contraction. Such effects could involve multiple mechanisms, including anticholinergic activity, β-adrenergic receptor stimulation, and activation of ATP-sensitive potassium channels [11]. Conversely, Cheng et al [28] reported that curcumin increased muscle tone in isolated urinary bladder preparations, potentially through the activation of local M1 muscarinic receptors (M-1-mAChR) linked to PLC-PKC pathways. These contrasting findings underscore the complex and context-dependent effects of curcumin and its derivatives on smooth muscle function. Moreover, curcumin has been reported to exert anti-inflammatory effects through suppression of NF-κB signaling and subsequent downregulation of pro-inflammatory mediators, including TNF-α, IL-1β, COX-2, and iNOS [29–31]. A previous study has suggested that curcumin may modulate cholinergic/muscarinic-related signaling and bladder smooth muscle activity, potentially contributing to the restoration of bladder dysfunction [32]. In addition, curcumin has also been shown to activate cholinergic anti-inflammatory pathways and attenuate cytokine production in animal and in vivo models of inflammation [33, 34].

One limitation of the present study is the lack of evaluation of inflammatory cytokines, histopathological alterations, and oxidative stress markers, which could provide additional mechanistic support for the observed protective effects. Further investigations incorporating these parameters are needed to better elucidate the underlying anti-inflammatory and antioxidant mechanisms. Further investigations are warranted to clarify the molecular mechanisms through

which HWC exerts its uroprotective effects and to determine its potential therapeutic applicability in bladder overactivity. In addition, future studies incorporating female animals will be important to assess whether the observed effects of HWC are reproducible across sexes and clinically relevant to OAB conditions in females. To our knowledge, this is the first study to demonstrate the beneficial effect of HWC in attenuating CYP-induced bladder overactivity in mice. The improvement in voiding dysfunction and muscarinic-related signaling observed following HWC treatment may involve restoration of bladder signaling pathways associated with attenuation of inflammatory processes, rather than direct muscarinic receptor antagonism. Nevertheless, further studies incorporating inflammatory and receptor-specific analyses are required to elucidate the precise mechanisms underlying the effects of HWC. These findings provide novel insight into the development of HWC, derived through an environmentally friendly preparation process, as a promising alternative therapeutic candidate for the management of OAB.

CONCLUSION

Oral administration of HWC (50 mg/kg) exerted a protective effect against CYP-induced bladder overactivity in mice by reversing the enhanced muscarinic responsiveness of the bladder. Further investigations are warranted to determine the therapeutic window and to elucidate the underlying mechanisms through which HWC ameliorate bladder overactivity.

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