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The Uroprotective Efficacy of Total Ginsenosides in Chinese Ginseng on Chemotherapy with Cyclophosphamide

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Abstract: Hemorrhagic cystitis is a recognizable complication of cyclophosphamide (CYP) attributable to its lively metabolite acrolein, which produces urothelial injury. The study intended to examine the uroprotective efficacy of total ginsenosides in Chinese ginseng (TGCG) in CYP-induced hemorrhagic cystitis. In total, 24 virgin female rats were randomized into four groups as follows: group 1 (control group; injected with normal saline), group 2 (injected with CYP plus a placebo with normal saline), group 3 (given CYP and TGCG (200 mg/kg)), and group 4 (given CYP and 2-mercaptoethane sulfonate sodium (Mesna, 30 mg/kg)). An evaluation by cystometry was conducted. Values of the voiding interval were assessed in anesthetized rats and histological examinations of the bladders were measured. In the cystometry analysis, the voiding interval was significantly reduced in the CYP group. TGCG and Mesna significantly increased in the voiding interval values, individually. Bladder edema and urothelial injury were examined after contact with CYP. Contrasted to the group given CYP, CYP-induced hemorrhagic cystitis, TGCG significantly increased the urothelial thickness, and significantly reduced scores of mucosal break and submucosal edema in the bladder. In conclusion, these findings mean that the treatment with TGCG in CYP rats can avoid hemorrhagic cystitis. TGCG decreases urothelial injury. TGCG may participate as the chief character of uroprotection in CYP-induced hemorrhagic cystitis.

Keywords: cyclophosphamide; uroprotection; urothelial damage; total ginsenosides in Chinese ginseng

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1. Introduction

Cyclophosphamide (CYP) is a cytotoxic alkylating agent that is operated in the chemotherapeutic treatment of definite solid tumors [1], lymphoproliferative disorders, and non-neoplastic diseases for example rheumatoid arthritis, systemic lupus erythematosus, and nephrotic syndrome [2]. Hemorrhagic cystitis, depicted as a symptomatic event (specified burning sensation, dysuria, and urinary frequency) of microscopic or macroscopic hematuria [3], is a familiar unfavorable effect of CYP and may therefore establish a restrictive factor in its use [4,5].

In the lack of sufficient uroprotection, the incidence of the side effect of CYP therapy as mentioned earlier varies from 2 to 40% in patients taking low doses of CYP on a long-term foundation [6]. Mortality has been stated to differ between 2 to 4% in patients with massive hemorrhage, who were given a high dose of intravenous CYP [7]. Acrolein, a urotoxic metabolite of CYP emerges to be liable for bladder injury. It has been proposed that urothelial injury happens by direct contact of the bladder with acrolein, which causes edema, ulceration, neovascularization, hemorrhage, and necrosis [8]. Given the restricted obtainability of human tissue for research, animal models could supply a major helper to better our comprehension of CYP-induced hemorrhagic cystitis.

2-mercaptoethane sulfonate sodium (Mesna) includes a sulfhydryl group that attaches acrolein, thus detoxifying the acrolein inside the urinary tract; the subsequent inert thioether is produced harmlessly in the urine without causing any uroepithelium injury [9]. Mesna could efficiently provide the wanted uroprotection, but CYP-related hemorrhagic cystitis still happens in patients (10 to 40%) with Mesna treatment [10]. Common Mesna side consequences comprise vomiting, headache, loss of appetite, sleepiness, cough, joint pain, and rashes. Furthermore, important side consequences contain anaphylaxis among others [11].

The most satisfactory theory on the pathogenesis of CYP-related hemorrhagic cystitis is urothelial injury, and hemorrhage, both of which could cause the activation of critical mediators of cystitis such as cytokines and nitric oxide (NO) [12,13]. This theory causes the hypothesis that the disease can be stopped with a reduction in the level of NO. Findings from related literatures disclosed that, urothelial injury is the dominant subject of CYP-induced hemorrhagic cystitis [8,12,13]. For this reason, the expansion of useful therapeutic agents for uroprotection are new therapeutic plans. These agents include Chinese medicinal herbs, which can lead to the wanted uroprotective effect while safely avoiding the appearance of the side consequences of Mesna as cited earlier.

Current literatures indicated that *Panax ginseng* Meyer has several pharmacological advantages, including anti-oxidant, anti-tumor [14], anti-inflammatory, anti-microbial, anti-diabetic, and anti-aging activities [15]. Our previous report revealed that ginsenoside Rh2 (one of the major bioactive ginsenosides from *Panax ginseng*) meaningfully improved the physiological signs of vaginal distension-induced stress, urinary incontinence, and decreased blood pressure; the ginsenoside Rh2 may consequently induce the wanted therapeutic effect in stress-induced urinary incontinence [16]. Chemotherapy-related side consequences influence the life quality of cancer patients. Current approaches for treating chemotherapy-related side consequences are poorly effective. Thus, creating effective new agents derived from non-toxic natural compounds for chemotherapy-related side consequences is necessary. Current studies specify that *Panax ginseng* ginsenosides may be an effective and non-toxic option for the chemotherapy-related side consequences. The mechanisms concentrate on anti-apoptosis, anti-inflammatory, and anti-oxidative pathways [17]. In addition, the extracted total ginsenosides in Chinese ginseng (TGCG) from the leaves and stem own a dammarane skeleton that has been shown to have a notable potential for producing numerous biological results [18,19]. TGCG could have potential in the treatment of diabetes through its anti-oxidant result, thus recovering insulin sensitivity [18,20]. Previous studies indicated that CYP-induced hemorrhagic cystitis can be stopped by numerous anti-oxidative and anti-inflammatory agents [21,22]. Therefore, the uroprotective efficacy of TGCG in CYP-induced hemorrhagic cystitis was studied.

2. Materials and Methods

2.1. Ethical Statement

The protocol of this study was approved by the Institutional Animal Care and Use Committee of the China Medical University, Taichung, Taiwan (Protocol No. CMUIACUC-2019-129-1, 17 February 2020).

2.2. Chemicals and Reagents

CYP were purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). The extracts of TGCG (Batch No. ZL180412) were purchased from Fusol Material Co., Ltd. (Tainan, Taiwan). Mesna were purchased from Sigma-Aldrich (St. Louis, MO, USA). Urethane was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hematoxylin and eosin (H&E) Staining Kit was purchased from Abcam (Boston, MA, USA). Unless otherwise specified, all reagents were from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Generating CYP-Induced Hemorrhagic Cystitis in Rat with TGCG

CYP is frequently used in the treatment of gynecological cancer which may cause hemorrhagic cystitis. Thus, the experiments were performed on female rats in the present study [21,23]. The dosages of the extract and compounds were chosen on the basis of literature data [18,24] and our pilot study. Virgin female Sprague-Dawley rats (225–250 g, 6–8 weeks, BioLASCO Co., Ltd., Taipei, Taiwan) were randomized into four groups (n = 6 for each group): group 1 (control group; injected with normal saline), group 2 (injected with CYP plus a placebo with normal saline), group 3 (given CYP and TGCG (200 mg/kg)), and group 4 (given CYP and Mesna (30 mg/kg)). First, virgin female rats in groups 2, 3, and 4 were given saline, Mesna, or TGCG for 7 days. CYP-caused urothelial damage was induced by a single dose of 150 mg/kg CYP (melted in normal saline; intraperitoneal (i.p.)) [25,26]. Control rats received saline injections. The experiments were performed 24 h later after injection.

2.4. Suprapubic Tube Implantation

An implantation suprapubic tube (SPT; PE-10 tubing, Clay Adams, Parsippany, NJ, USA) was introduced into the bladder. Major points of the operation include: (1) a midline longitudinal abdominal incision was made (50 mm above the urethral meatus); (2) a tiny incision was created in the bladder wall, and SPT tubing with a flared tip was introduced in the bladder dome; and (3) the purse-string suture of 8-0 silk was tightened around the catheter, which was excavated subcutaneously to the neck, where it left the skin. The procedure of surgery was conducted under 1 g/kg urethane anesthesia (i.p.) according to the procedures previously described [27–30].

2.5. Cystometry

A syringe pump and a pressure transducer were connected to the bladder catheter. The rats were put supine at zero-level pressure while bladders were fulfilled with saline (20 μL/min) through the bladder catheter. After a 30 min equilibration period, intravesical pressure was recorded for 30 min [31]. Ten samples per second of the computer data (PowerLabs, ADInstruments, Bella Vista, Australia) for the pressure and force transducer signals were amplified and digitized for data collection.

2.6. Histological Analyses

The rats were sacrificed directly after cystometry, and the bladders were gathered [32,33]. The tissue samples were fixed in formalin for H&E stain. The specimens were examined and photographed under light microscopy and [34–36].

The histologist was blinded to the groups and the severity of the mucosal break, submucosal edema, and hemorrhage was scored in 10 fields of each slide. No break, edema, and hemorrhage were scored as 1, minimal as 2, moderate as 3, and severe as 4.

2.7. Statistical Assay

The data were assessed by Student's *t*-test or analysis of variance. All statistical tests were two-sided. All calculations were performed using the Statistical Package for Social Sciences (SPSS for Windows, release 28.0, SPSS Inc., Chicago, IL, USA). *p*-value less than 0.05 was decided statistically significant.

3. Results

3.1. Value of the Voiding Interval

While the value of the voiding interval (the time between voids) obtained in group 2 (CYP group) was significantly decreased as contrasted to that of the control group (group 1), values of voiding interval significantly increased in groups 3 (CYP + TGCG group) and 4 (CYP + Mesna group), respectively, contrasted to group 1 (Figure 1).

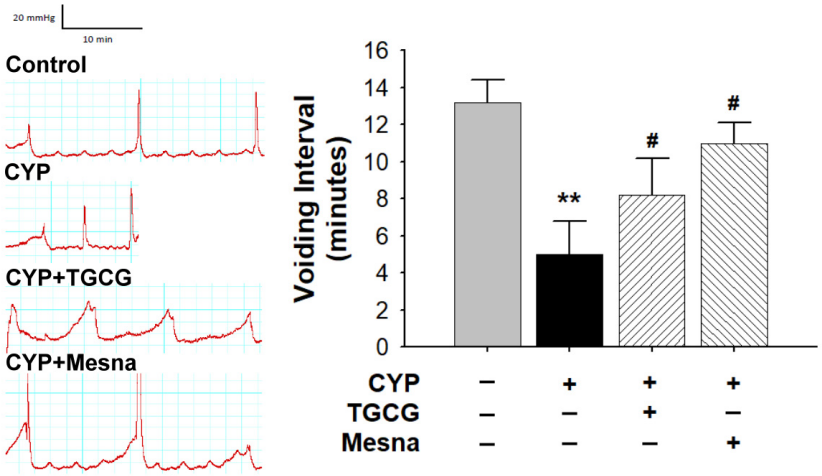

Figure 1. Cystometry traces between group 1—saline-injected control, group 2—CYP + placebo with normal saline, group 3—CYP + TGCG (200 mg/kg), and group 4—CYP + Mesna (30 mg/kg). In the cystometry assay, the peaks signify the detrusor contraction. Voiding interval values were presented in the different groups. Each bar in the figure denotes the mean ± standard deviation. ** $p < 0.01$ contrasted to control group. # $p < 0.05$ contrasted to CYP.

3.2. Average Weight of Bladder

Compared to the control group, the average weight of the bladder in the CYP group significantly increased. However, only the average weight of the bladder in group 4 (CYP + Mesna group) significantly reduced as contrasted to that in the CYP group (Figure 2).

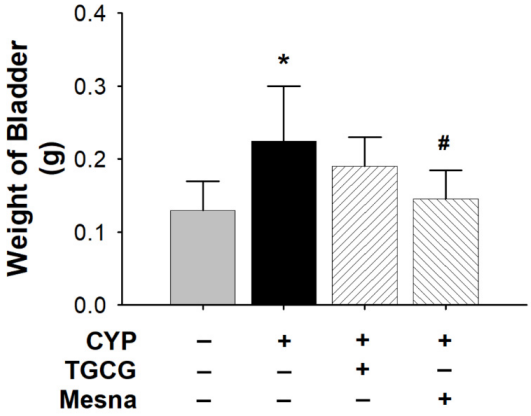

Figure 2. Average weight of bladders between group 1—saline-injected control, group 2—CYP + placebo with normal saline, group 3—CYP + TGCG (200 mg/kg), and group 4—CYP + Mesna (30 mg/kg). Each bar in the figure denotes the mean ± standard deviation. * $p < 0.05$ contrasted to control group. # $p < 0.05$ compared to CYP.

3.3. Histological Analyses for the Cross Sections of Bladders

A higher magnification of the pictures (Figure 3) presented that the normal urothelium is lined with transitional epithelium. After exposure to CYP, it displayed that most

superficial layers of cells were damaged. Contrasted to the control group, in the bladder, urothelial thickness in the CYP-treated group significantly reduced while the CYP-induced hemorrhagic cystitis, and the urothelial thickness in the bladder of group 3 (CYP + TGCG group) increased (Figure 4). Contrasted to the control group, submucosal thickness in the bladder of the CYP-treated group significantly increased while the CYP-induced hemorrhagic cystitis, and the submucosal thickness in the bladder of group 4 (CYP + Mesna group) decreased (Figure 4).

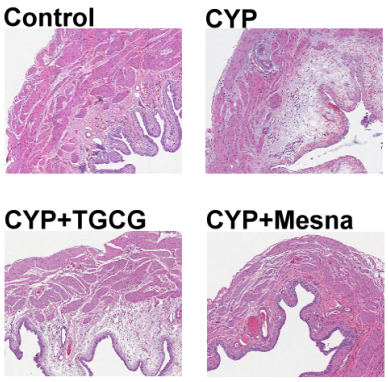

Figure 3. H&E stain for histological examination in bladder cross sections between control, CYP, CYP + TGCG, and CYP + Mesna groups in the female rats.

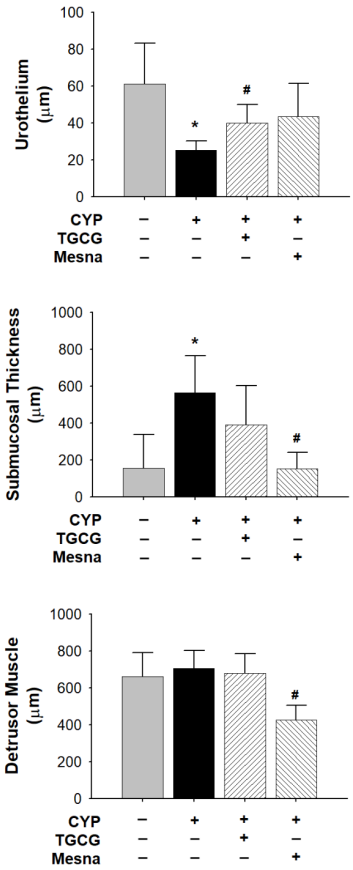

Figure 4. Average thickness of histological layers of the bladder between group 1—saline-injected control, group 2—CYP + placebo with normal saline, group 3—CYP + TGCG (200 mg/kg), and group 4—CYP + Mesna (30 mg/kg). Each bar in the figures denotes the mean ± standard deviation. * $p < 0.05$ contrasted to control group. # $p < 0.05$ contrasted to CYP group.

3.4. Histological Analyses of Bladder Wall

Contrasted to the control rat, the scores of the mucosal break in the bladder of the CYP group meaningfully increased while CYP-caused hemorrhagic cystitis, and the scores of the mucosal break in the bladder of groups 3 (CYP + TGCG group) and 4 (CYP + Mesna group) significantly reduced. In addition, as contrasted to the control, the scores of submucosal edema in the bladder of the CYP group significantly increased while CYP-caused hemorrhagic cystitis, and the scores of submucosal edema in the bladder of groups 3 (CYP + TGCG group) and 4 (CYP + Mesna group) significantly reduced. However, while the scores of hemorrhage in the bladder of the CYP group were significantly increased contrasted to the control group, no significant reduction were examined in the bladder of groups 3 (CYP + TGCG group) and 4 (CYP + Mesna group) (Figure 5).

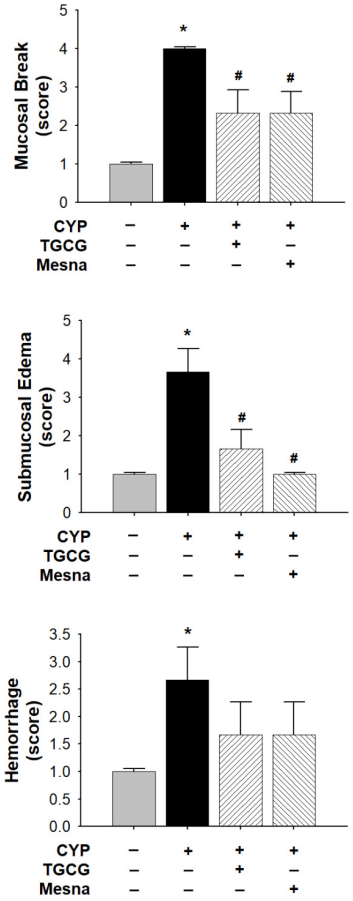

Figure 5. Histological analyses of bladder wall as signified by mucosal break, edema, and hemorrhage between group 1—saline-injected control, group 2—CYP + placebo with normal saline, group 3—CYP + TGCG (200 mg/kg), and group 4—CYP + Mesna (30 mg/kg). Each bar in the figures denotes the mean ± standard deviation. * $p < 0.05$ compared to control group. # $p < 0.05$ compared to CYP.

4. Discussion

Hemorrhagic cystitis is an accepted complication of CYP (attributable to its active metabolite acrolein), which produces urothelial injury via key mediators such as cytokines and NO. In this study, urothelial injury was examined after exposure to CYP. Our findings suggest that the urothelial injury might cause classic signs of a leaky barrier, which may cause urinary frequency in the rats with CYP-induced hemorrhagic cystitis. Values of voiding interval were examined to be significantly increased in the TGCG and Mesna groups. Since chemotherapy-related side effects affect the life quality of cancer patients, developing effective new agents derived from non-toxic natural compounds for chemotherapy-related side effects is necessary. Current studies indicate that *Panax*

ginseng ginsenosides may be an effective and non-toxic option for chemotherapy-related side effects [17]. These findings suggest that TGCG and Mesna, respectively, could be productive in the prophylaxis of CYP-caused hemorrhagic cystitis [37]. The potential anti-inflammatory and anti-oxidative mechanisms of TGCG might contribute to their reversing effects on CYP-induced changes [17].

Contrasted to the group—CYP-induced hemorrhagic cystitis, the scores of mucosal break and submucosal edema in the bladder of group 3 (CYP + TGCG) were examined to have significantly reduced. These findings proposed that TGCG via its anti-oxidant effect could reduce urothelial injury, thus lessening urinary frequency in rats treated with CYP. Furthermore, there is proof that TGCG could participate in a main uroprotective role in CYP-induced urothelial injury.

CYP has clinical uses with a wide range as an immunosuppressive and anti-cancer agent, although there are currently no accessible reliable replacements that have been approved for clinical uses in the market. Since there the use of CYP is unavoidable, the study focused on lessening the side effects of CYP by protecting various important non-targeted tissues, including the bladder [38]. Jang et al. assessed the effects of ginseng saponin on bladder relaxation and found that the saponin of ginseng caused a dose-dependent relaxant effect on the urethra strips significantly in the rabbits. The change in urethral perfusion pressure between relaxation and baseline was significantly raised in the ginseng saponin group than in the control group. Therefore, on the bladder, a notable relaxation effect of saponin was examined. The mechanism by which ginseng saponin causes relaxation is unclear, however, it might be disclosed to involve the NO pathway [39].

The relief of the CYP side effects is a prominent parallel therapy in synergistic anti-cancer drug use. Recently, various animal models offer a very genial alternative for humans to fulfill the toxicological research of chemotherapeutic drugs [40]. The effects and mechanisms of ginseng on other CYP-related side effects in multiple organ systems have also been studied. Han et al. examined, and compared the effects, and underlying mechanisms of major components of ginseng on CYP-induced myelosuppression. The result gained showed that the major components of ginseng could improve the hematopoietic function in the CYP-induced myelosuppressed mice [41]. Abdelfattah-Hassan et al. obtained that ginseng elicits potent hepatoprotective effects, and should therefore be considered in a case of possible liver injury secondary to damaging medications such as CYP [42]. Hosseini et al. assessed the ginseng effects on CYP-caused testicular toxicity, and confirmed the helpful effects of ginseng on CYP-induced reproductive toxicity of male rats [43].

Natural products have attracted scientists' attention for their medical applications. For example, *Centella asiatica* represents a medicinal herb that may be a source of several natural agents. One instance of these chemical substances is asiatic acid—an aglycone form of asiaticoside. The activity and potential role of asiatic acid in the treatment of various chronic disorders are due to its commercial availability, pharmacological properties, and low toxicity. Wróbel et al. had found that asiatic acid can be used as an effective agent in the rat model of CYP-caused cystitis, reversing the inflammatory responses and restoring the lower urinary tract functional properties. Asiatic acid also markedly decreased bladder edema and urothelium thickness [21]. Moreover, GPR55 (a novel cannabinoid receptor) agonists are a novel class of uroprotective agents, focusing on inflammatory cystitis. Wróbel et al. also reported that a potential candidate O-1602 (GPR55 agonist) prevented urinary complications with chemotherapy. The beneficial medical effects of CYP-caused cystitis may be via anti-inflammatory and anti-oxidant mechanisms, leading to various histological and cytometric property changes [22].

However, our study has several limitations: (1) Different from human females, the pelvic floor structure of virgin female mice is quadrupedal with a loose abdominal wall [44]. Therefore, the results from this present study need to be carefully used in humans [33,45]; (2) Integral bladder cystometry profiles such as basal pressure, intercontraction interval, bladder compliance, detrusor overactivity index, non-voiding contractions (NVCs), volume threshold to elicit NVC, voided volume, and post-void residual could help us monitor the

bladder function comprehensively [46]. Moreover, urodynamic analyses were performed under anesthesia [47]; (3) In the animal study, the effects of TGCG on proliferation, differentiation, and maturation of urothelial cells were not independently investigated in vitro. Additionally, Mesna was found to be superior in many of our experiments. However, common Mesna side effects include vomiting, headache, loss of appetite, sleepiness, cough, joint pain, and rashes; momentous side effects include anaphylaxis, among others. In contrast, TGCG are natural pharmaceutical ingredients characterized by a complex composition with fewer side effects [11,48,49], TGCG might be inferior but we expect lower toxicity. Nevertheless, there were no data regarding the side effects in our experiments; (4) Recent studies have focused on the effects of natural anti-inflammatory agents such as TGCG, largely due to their relative reliability, affordability, and safety [50]. However, we did not further design the TGCG-only or TGCG combination with Mesna groups in the present study. It would be advisable to see if there is any toxic effect of TGCG. The uroprotective mechanism of TGCG in CYP-induced hemorrhagic cystitis was not analyzed in this study; and (5) More research to convert these findings into novel therapeutics, for women on chemotherapy of CYP, are ensured for further clarity.

5. Conclusions

The study investigated the uroprotective efficacy of TGCG in CYP-induced hemorrhagic cystitis. In the cystometry analysis, TGCG and Mesna meaningfully increased in the voiding interval values, respectively. Bladder edema and urothelial injury were observed after exposure to CYP. Contrasted to the group treated with CYP, TGCG significantly increased the urothelial thickness, and significantly reduced scores of mucosal break and submucosal edema in the bladder. These findings suggest that the treatment with TGCG in CYP rats can prevent hemorrhagic cystitis. TGCG may play the potential role of uroprotection in CYP-induced hemorrhagic cystitis.

Author Contributions: Conceptualization, H.-Y.C.; methodology, Y.-H.C., W.-C.C., S.-J.C., S.-J.W., P.-L.L., M.-Y.T., C.-T.L., D.-C.C. and H.-Y.C.; software, Y.-H.C., S.-J.W. and H.-Y.C.; validation, Y.-H.C., W.-C.C., S.-J.C., S.-J.W., P.-L.L., M.-Y.T., C.-T.L., D.-C.C. and H.-Y.C.; writing—original draft preparation, Y.-H.C. and H.-Y.C.; writing—review and editing, Y.-H.C. and H.-Y.C.; supervision, H.-Y.C.; project administration, H.-Y.C.; funding acquisition, Y.-H.C. and H.-Y.C.; Y.-H.C. and D.-C.C. contributed equally to this study. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The protocol was approved by the Institutional Animal Care and Use Committee of the China Medical University, Taichung, Taiwan (Protocol No. CMUIACUC-2019-129-1, 17 February 2020).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be provided on request.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CYP	cyclophosphamide
H&E	hematoxylin and eosin
i.p.	intraperitoneal
NO	nitric oxide
NVCs	non-voiding contractions
SPSS	Statistical Package for Social Sciences
SPT	suprapubic tube
TGCG	total ginsenosides in Chinese ginseng

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