

1 **Therapeutic effects of traditional Chinese medicine formula Qianlie Tongli**
2 **decoction on chronic prostatitis/chronic pelvic pain syndrome induced by**
3 **peptide T2 in mice**

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21 **Abstract**

22 **Objectives:** This study was undertaken to reveal therapeutic effects and the preliminary

23 mechanism of Chinese medicine formula Qianlie Tongli decoction (QTD) in chronic
24 prostatitis/chronic pelvic pain syndrome (CP/CPPS).

25 **Methods:** A total of 50 male C57BL/6 mice were randomly divided into five groups. All
26 groups except the control group were injected subcutaneously T2 peptide emulsion, which
27 induced the CP/CPPS model. After the induction of CP/CPPS, the model group was given 0.9%
28 NaCl by oral gavage while low-dose, medium-dose, and high-dose groups were treated with
29 Chinese medicine formula. Micturition habits and pain behavior of mice were analyzed for
30 each group. Hematoxylin and eosin staining were used to investigate prostate inflammation.
31 The serum level of tumor necrosis factor- α (TNF- α) was measured by enzyme-linked
32 immunosorbent assay (ELISA) kit.

33 **Key findings:** Chinese medicine formula significantly reduced the number of urine spots and
34 improved pain response frequency in the medium-dose and high-dose group. The high-dose
35 group showed reduced considerably inflammatory lesion and inflammatory cell infiltration
36 than the low-dose and medium-dose groups. Serum levels of TNF- α in the high-dose group
37 were significantly reduced compared with the model group.

38 **Conclusions:** The results demonstrated the therapeutic effects of Qianlie Tongli decoction in
39 CP/CPPS mice by analyzing clinically relevant symptoms (urinary tract system, pelvic pain,
40 and prostate inflammation), and preliminary explored the inflammatory-related treatment
41 mechanisms by measuring TNF- α .

42 **Keywords:** Qianlie Tongli decoction; Chronic prostatitis/chronic pelvic pain syndrome
43 (CP/CPPS); Anti-inflammation; Therapeutic effects; Immunization.

44

45 1. Introduction

46 According to the National Institutes of Health (NIH), prostatitis has acute bacterial prostatitis
47 (category I), chronic bacterial prostatitis (category II), chronic prostatitis/chronic pelvic pain
48 syndrome (category III) and asymptomatic inflammatory prostatitis (category IV) [1]. NIH
49 Category III Prostatitis is a highly extensive disease that affects men with a wide age range
50 and severely affects the quality of life (QoL) [2]. Besides, population-based surveys have
51 shown that CP/CPPS is as severe as Crohn's disease, diabetes, and heart failure [3]. As the
52 most prevalent type of prostatitis, almost 90% to 95% of men have CP/CPPS with symptoms
53 of chronic prostatitis [4]. CP/CPPS is mostly described by long-term, repeated pelvic pain or
54 discomfort, lasting more than 3 months, with sexual dysfunction and variable urinary
55 symptoms [5].

56 Presently, etiology and pathophysiology are not well-known. Many scholars believe that it
57 might be related to pathogen infection, mental and psychological factors, neuroendocrine,
58 immune function, and oxidative stress [6, 7]. Autoimmunity is a crucial factor in CP/CPPS,
59 previous studies have shown that autoimmune prostatitis exists in human males, and immune
60 disturbance leads to the loss of self-tolerance to prostatic antigens [8, 9].

61 Currently, there are no established treatments to alleviate symptoms for CP/CPPS. The
62 therapeutic interventions for CP/CPPS mainly include alpha-blockers, antibiotics, pain
63 medications, and multimodal therapy [10-12]. Weak evidence supports the use of α -blockers,
64 pain medications, and a four to six weeks course of antibiotics for the treatment of CP/CPPS
65 [13]. In China, for the treatment of numerous diseases, herbal has been widely used. Some
66 studies provided evidence that herbal supplements could be effective in CP/CPPS [14].

67 Herbal can improve the clinical symptoms, reverse some pathological changes, and restore
68 the body's normal physiological function. For the treatment of CP/CPPS, there are several
69 approaches in herbal, including alleviating pain and dispersing liver-qi, removing dampness,
70 and clearing heat, and activating blood to dissolve stasis. Pro-inflammatory cytokines
71 expedite CP/CPPS development, while anti-inflammatory cytokines alleviate the disease [15].
72 Numerous researchers have shown that herbal protects against cytokine production in
73 atherosclerosis as the potential immunosuppressive agents [16]. Such diverse and flexible
74 therapies in Chinese medicine uncover the advantages of herbal treatment for CP/CPPS.
75 Qianlie Tongli decoction (QTD) is a Chinese medicine formula consisting of *Hedyotis diffusa*,
76 *Epimedium brevicornu* Maxim, *Fritillaria thunbergii*, Cortex Phellodendri Chinensis (CPC),
77 and Earthworm (*Lumbricus terrestris*). These Chinese medicinal materials can be used
78 individually or in combination. QTD has been used widely in the clinical setup in China for
79 the treatment of various inflammatory conditions. Previous studies have revealed the
80 anti-inflammatory effect of *Hedyotis diffusa*, *Epimedium brevicornu* Maxim, *Fritillaria*
81 *thunbergii*, CPC, and Earthworm (*Lumbricus terrestris*) individually [17-21]. In this
82 experiment, QTD takes "Bushen Tongluo" as the treatment principle, and *Epimedium* is the
83 primary medicine to nourish the kidney and remove dampness in this formula. *Hedyotis*
84 *diffusa* and *Fritillaria thunbergii* are the medicines for clearing heat and detoxifying. The
85 adjuvant medicine-Earthworm can promote blood circulation and facilitate the effects of
86 other medications. CPC and *Fritillaria thunbergii* used to disperse blood stasis and achieve
87 the anti-inflammatory effect. Previously for the first time, we have evaluated the effects of
88 QTD formula in an autoimmune prostatitis rat model [22]. In this study, we investigated the

89 therapeutic effects of Chinese medicine formula QTD for CP/CPPS mice and explored the
90 preliminary mechanism.

91 **2. Method and materials**

92 The main reagent used in this experiment was T2 peptide with the sequence of CSEEM
93 RHRFR QLDTK LNDLKG amino acid from Transient receptor potential cation channel
94 subfamily M member 8 (TRPM8), it is an epitope of antigenic nature inducing experimental
95 autoimmune prostatitis (EAP) was purified and synthesized by Wuhan Buyers Biotechnology
96 Co., Ltd., China. Complete Freund's adjuvant (CFA) was obtained from the Sigma-Aldrich
97 Chemical Co. (St. Louis, MO, USA). Chinese medicine compounds included *Hedyotis diffusa*,
98 *Epimedium brevicornu* Maxim, *Fritillaria thunbergii*, CPC, and Earthworm (*Lumbricus*
99 *terrestris*), which were purchased from Nanjing Crane Age Pharmaceutical Service Co., Ltd.,
100 China (Batch #: Su Cai Zhunyin (2017) No. 019-022). The four voucher specimens were
101 preserved at the herbarium of China Pharmaceutical University, and the materials for formula
102 were authenticated by Prof. Dr. Zhang Chunfeng, College of Chinese Medicine, China
103 Pharmaceutical University [*Fritillaria thunbergii* (Liliaceae), CPU180910-1; *Epimedium*
104 *brevicornu* Maxim (Berberidaceae), CPU180910-2; *Hedyotis diffusa* (Rubiaceae),
105 CPU180910-3; Cortex *Phellodendri Chinensis* (Rutaceae), CPU180910-4; Earthworm
106 (*Lumbricus terrestris*), CPU180910-5].

107 **2.1. Ethical Statement**

108 All procedures and experiments used in this study comply with guidelines of the local
109 institutional ethical committee of the China Pharmaceutical University (Jiangsu Province,
110 Nanjing, China) regarding care and welfare of animals under study (T/CPU, 235-2018,

111 2018-09-09).

112 **2.2. Animal model**

113 Fifty adult male 6-8 weeks old C57BL/6 mice weighing 18~22g were obtained from
114 Qinglong Mountain Animal Breeding Ground (Nanjing, China). These mice were kept and
115 fed under standard temperature and relative humidity with a 12-h light/dark cycle in an
116 animal room. The ethical committee approved animal handling and experimental procedures
117 of China Pharmaceutical University. These mice were randomized into five groups (n=10),
118 including the control, model, low-dose, medium-dose, and high-dose group.

119 **2.3. Immunization**

120 T2 peptide was dissolved to 1mg/ml of reserves for a combination of an equal concentration
121 of CFA before immunization [23]. Except for the control group, the other groups were all
122 given a multipoint subcutaneous injection on days 0 and 14. Every time, mice were injected
123 with 200 μ l of a mixture subcutaneously with five injections separately in the back. For the
124 mice, the ultimate concentration of T2 peptide was 225 μ g/ml.

125 **2.4. Preparation and administration of QTD**

126 The composition of TCM formula QTD is as follows: *Hedyotis diffusa* (60g), *Epimedium*
127 *brevicornu* Maxim (60g), *Fritillaria thunbergii* (60g), CPC (60g), and Earthworm (60g)
128 (Table 1). Based on the traditional Chinese herbal decoction method, these five agents were
129 mixed and kept for 1 hour in the arenaceous pot with eight volumes of cold water. Then the
130 mixture was boiled for 40min and filtered. The first filtered solution was then reserved, and
131 formula materials were again mixed in six volumes of cold water, boiled for 40 min and
132 filtered. The second filtered solution was then reserved, and then in four volumes of cold

133 water, the formula materials were mixed again, boiled for 40 min and filtered. The three filter
 134 liquors were combined and concentrated 0.5g/ml, 0.75g/ml, and 1.0 g/ml for the low, medium,
 135 and high-dose groups, respectively. After the development of the CP/CPPS model on day 28,
 136 based on previous methodology [24, 25], each mouse in the low-dose, medium-dose, and
 137 high-dose group received 0.15ml/10g daily by oral gavage between the day 29 and 56.

Scientific name	Latin binomial	Chinese name	Part used	Weight(g)	Voucher #
<i>Fritillaria thunbergii</i>	<i>Fritillaria thunbergii</i>	Zhebeimu	Bulb	60g	CPU180910-1
<i>Epimedium brevicornu</i> Maxim	<i>Epimedium Folium</i>	Yinyanghuo	Leaf	60g	CPU180910-2
<i>Hedyotis diffusa</i>	<i>Oldenlandia diffusa</i>	Baihuasheshecao	Whole plant	60g	CPU180910-3
Cortex Phellodendri Chinensis (CPC)	<i>Phellodendri Chinensis</i>	Huangbo	Bark	60g	CPU180910-4
<i>Lumbricus terrestris</i> (Earthworm)	<i>Lumbricidae terrestris</i>	Dilong	Whole Body	60g	CPU180910-5
Total amount				300g	

138 **Table 1.** The composition of TCM formula QTD.

139 **2.5. Voiding Behavior Analysis**

140 Changes of urine spots were analyzed by voiding the spot assay (VSA) test [26].
 141 Experimental mice were placed on the filter paper in a cage individually. After one hour, total
 142 filter papers were collected, and the images of urine spots were taken under the ultraviolet

143 light. Then the urine spots of mice were evaluated by the Fiji version of ImageJ software. The
144 number of urine spots represents the urine frequencies, and the spots with size $\geq 6.6\text{mm}^2$
145 were calculated. Blinded observers collected data.

146 **2.6. Pain Threshold Assessment**

147 Mice were tested on days 28 and 56 by using von Frey filaments (vFF) applied to the
148 abdomen and the plantar region of the hind paw. Referred hyperalgesia and tactile allodynia
149 were tested. The technique was done in stainless steel chambers. Ten fibers with forces of
150 0.04, 0.07, 0.16, 0.40, 0.60, 1.00, 1.40, 2.00, 4.00 and 6.00 of consistently increasing weights
151 were exerted to pelvic regions, and withdrawal responses of mice were evaluated. Each
152 filament was applied for 1~2s with a 5s interval for a total of 10 times, and in ascending order
153 of force, the hairs were tested. Immediate licking or scratching of the area of filament
154 stimulation, sharp retraction of the abdomen, and jumping of mice were measured as a
155 positive response to fiber stimulation. Response frequency was considered as the mean
156 percentage of positive responses.

157 **2.7. Histopathology**

158 Prostate tissues from mice were soaked in 10% PFA for 24-48 hours. The fixed tissues were
159 dehydrated in different solvents and fixed in paraffin. After that, the samples were sliced into
160 sections of $5\mu\text{m}$, which were stained with H&E staining and examined under a microscope.
161 The severity of prostate tissue inflammation was assessed by using four-point scores in a
162 random double-blind method. Grade 0 means no inflammation, and the grade 1 means part of
163 acinar epithelial cell detachment and slight focal infiltration, the grade 2 means most of the
164 acinar epithelial cell detachment, epithelial cell necrosis, moderate focal and mild diffuse

165 infiltration and the grade 3 means most of the acinar epithelial cell detachment, epithelial cell
166 necrosis and severe diffuse infiltrate [27].

167 **2.8. Enzyme-linked immunosorbent assay (ELISA)**

168 To examine the anti-inflammatory effects of QTD, the TNF- α level in the serum was
169 identified by ELISA. The blood plasma obtained from the heart was put on room temperature
170 for 2 hours; it was then centrifuged at 3000 rpm for 10 min. After that, the supernatant was
171 collected and kept at -80°C. The content of TNF- α was calculated by using the ELISA kit
172 (Elabscience Biotechnology Co., Ltd., China) according to the manufacturer's protocol.

173 **2.9. Statistical analysis**

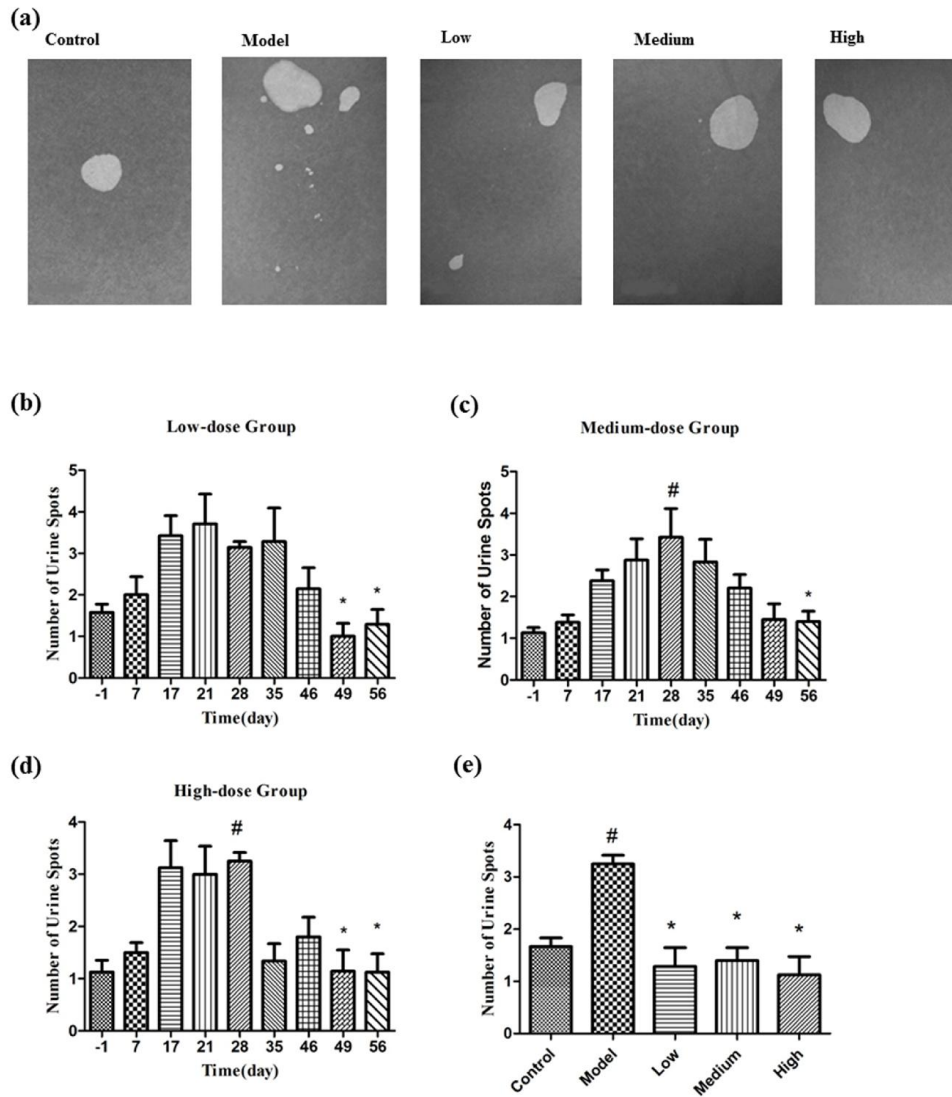
174 The statistical differences between control, model, and treatment groups were analyzed by
175 analysis of variance (ANOVA) analysis and expressed as mean, standard deviation (\pm SD). In
176 all the analyzes, $p < 0.05$, $p < 0.01$, $p < 0.001$ was considered statistically significant.

177 **3. Results**

178 **3.1. Voiding behavior analysis**

179 As shown in (**Fig. 1a**), urine spots were collected on filter paper, and a VSA test was used to
180 analyze the voiding behaviors. Several urine spots on day 28 were significantly higher as
181 compared to the day -1 and day 56 (**Fig. 1b-d**). After day 56, the number of urine spots was
182 significantly higher in the model group as compared to the control, the low-dose,
183 medium-dose, and high-dose group (**Fig. 1e**).

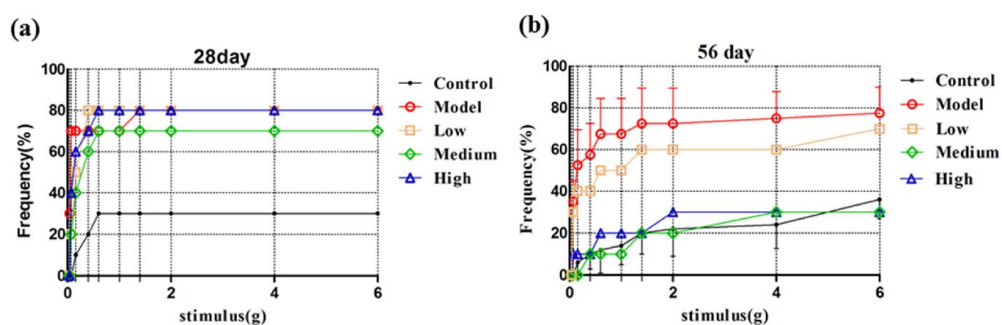
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185

186 **Fig.1a.** Analysis of voiding behavior using ImageJ software. Urine spots with size $\geq 6.6\text{mm}^2$ 187 were considered. **b, c, d, e.** Several urine spots. (b) *means $p < 0.05$ compared with day 21188 (c) #means $p < 0.05$ compared with day -1, *means $p < 0.05$ compared with day 28 (d) #means189 $p < 0.05$ compared with day -1, *means $p < 0.05$ compared with day 28 (e) *means $p < 0.05$ 190 compared with model group, #means $p < 0.05$ compared with control group.191 **3.2. Pain behavior analysis**

192 On day 28 of CP/CPPS induction and before the treatment with QTD, pain response
 193 frequency was recorded for all groups. On day 28th, maximum pain response frequency was
 194 observed in the model, low-dose, medium-dose, and the high-dose group (**Fig. 2a**) as
 195 compared to the control group. After the induction of CP/CPPS, the treatment groups
 196 received the treatment for 28 days, and the follow-up pain threshold on day 56 was compared
 197 to the day 28 pain threshold. On day 56, no significant difference was observed in the model
 198 and low-dose group, though the medium and high dose groups showed minimum pain
 199 response frequencies (**Fig. 2b**) as compared to day 28.



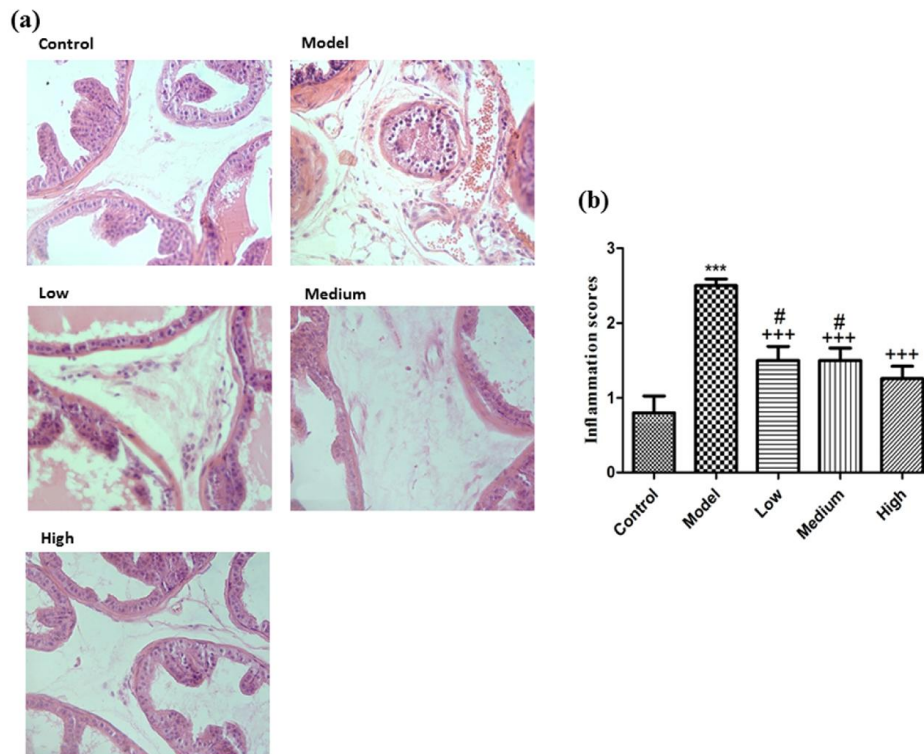
200

201 **Fig.2.** Pain withdrawal response frequencies in the model, low-dose, medium-dose, high-dose,
 202 and control group of mice after application of forces.

203 3.3. Histopathology

204 After Hematoxylin and eosin staining of the prostates, the model group appeared to have
 205 more severe inflammation than the control group. The low-dose group showed a large
 206 number of inflammatory infiltrations, while only scattered inflammatory infiltrates were seen
 207 in the medium-dose group. However, after the treatment, no significant inflammation and
 208 infiltration of inflammatory cells in a high-dose group were observed (**Fig. 3a**). The

209 inflammation score was most notable in the model group ($p<0.001$) compared to the control
 210 group. The low-dose, medium-dose, and high-dose group showed a significant difference
 211 ($p<0.001$) as compared to the model group. Compared to the control group, the low-dose and
 212 medium-dose groups were significantly different ($p<0.05$). The high-dose group showed no
 213 significant difference as compared to the control group. (**Fig. 3b**).



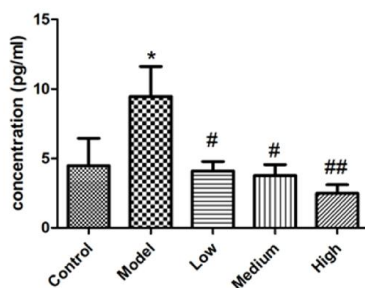
214

215 **Fig. 3a.** Observation of histopathology of the prostate tissue from C57BL/6 mice in the
 216 different groups (resolution: 400X). The model group appeared to have the most severe
 217 inflammatory lesion and the most extensive infiltration of inflammatory cells. The low-dose
 218 group showed a large number of inflammatory infiltrations. The medium-dose group
 219 displayed scattered inflammatory infiltrates. No significant inflammation and infiltration of
 220 inflammatory cells were observed in the high-dose group. The control group showed no

221 inflammation. **b.** Mean inflammation scores of each group. ***The most significant
222 difference from the control group at $p<0.001$. +++The most notable difference from the model
223 group at $p<0.001$. #Significant difference from the control group at $p<0.05$.

224 3.4. Serum levels of TNF- α

225 To detect the TNF- α level in the serum ELISA was used. As shown in **Fig. 4** and **Table 2**, the
226 serum level of TNF- α in the model group was significantly higher than the control group
227 ($p<0.05$). The low-dose and medium-dose groups showed no significant difference as
228 compared to the control group ($p>0.05$). The TNF- α levels in the high-dose group also
229 showed no significant difference from the control group ($p>0.05$). The high-dose group
230 showed the most significant difference ($p<0.01$) as compared to the model group. When
231 compared to the model group, the low-dose and medium-dose group showed a significant
232 difference ($p<0.05$).



233 **Fig. 4** Evaluation of the expression levels of TNF- α in serum. The ELISA kit detected levels
234 of TNF- α in blood serum. The model group showed increased levels as compared to the
235 control group ($*p<0.05$). The medium-dose group and the low-dose group showed a
236 significant difference ($#p<0.05$) as compared to the model group. The high-dose group
237 displayed the most significant differences ($##p<0.01$) as compared to the model group.

Group	TNF- α (pg/ml) mean \pm SD
Control	2.59 \pm 1.39
Model	9.46 \pm 6.81*
Low dose	4.10 \pm 1.53#
Medium dose	3.77 \pm 1.91#
High dose	2.47 \pm 1.53##

239 **Table 2.** TNF- α level in serum (means \pm SD). * p <0.05 means, compared with the control
 240 group. # p <0.05 indicates, comparison with the model group. ## p <0.01 means, comparison
 241 with the model group.

242 4. Discussion

243 CP/CPPS severely weakens the psychological and physical health of men. The etiology of
 244 this syndrome is not fully understood, and by western medicine, its treatment is frequently
 245 unsuccessful [28]. Traditional Chinese medicine is paying increasing attention to academic
 246 circles. Many scholars verified that TCM plays an essential role in reducing the risk of
 247 chronic disease [29]. Compared to the specific drug, the advantage of TCM therapeutics for
 248 CP/CPPS is “multiple components, multiple targets” to a complex disease[30]. CP/CPPS
 249 belongs to “Gonorrhea,” “Stranguria,” and “Turbid Semen.” The theories of TCM state that
 250 there are four kinds of common syndromes; downward flow syndrome of dampness-heat,
 251 gan-qi stagnation syndrome, blood-stasis syndrome, and deficiency of kidney-yang syndrome,
 252 which influence the treatment options [31-33]. The symptoms of damp heat and blood stasis
 253 are the most prevalent clinical syndrome, Wang Z's meta-analysis and literature review

254 demonstrate that TCM ranks highest in terms of improvement of CP/CPSS associated with
255 damp-heat and blood-stasis syndromes [30].

256 Qianlie Tongli decoction is composed of *Hedyotis diffusa*, *Epimedium brevicornu* Maxim,
257 *Fritillaria thunbergii*, CPC, and Earthworm (*Lumbricus terrestris*). QTD can clear heat,
258 remove dampness, and promote blood circulation. Nankang tablets are included in the
259 《Chinese Pharmacopoeia》, *Epimedium* is the main drug, and adjuvants include CPC and
260 *Hedyotis diffusa*. Icariin is the quality control indicator, Ni Yan's study [34] found that
261 berberine and oleanolic acid also as indicators. Xiang Dong's study [35] analyzed the
262 determination of icariine in Qianlietong tablets by HPLC; *Epimedium* is the main medicine in
263 Qianlietong tablets; the result suggests the icariine is the main active ingredient. Xie
264 Xueyuan's study [36] analyzed the determination of berberine in Qianlietong granules by
265 HPLC, CPC is the main medicine in Qianlietong granules, the negative control doesn't
266 contain CPC, the result suggests the berberine is the main active ingredient. 《Compendium
267 of Materia Medica》 and 《Shennong Materia Medica》 recorded that earthworm has the
268 functions of activating meridians, activating blood circulation and removing stasis.
269 Shuxuetong's main component is the earthworm, which can significantly improve the
270 symptoms of CP/CPSS [37]. Our lab found *Fritillaria thunbergii* can improve CP/CPSS mice
271 symptoms [18], so it as an adjunct. *Fritillaria* is insoluble in water, even if it may be major
272 active ingredients. So, we presumed that QTD' major ingredients were berberine, icariine, and
273 oleanolic acid. Due to a Chinese herbal formula has many active ingredients, which need
274 further research.

275 Numerous studies have shown that immune-related effects of TCM are associated with

276 cytokine regulation [38]. Inflammation may contribute to CP/CPPS. Many types of research
277 have shown that several pro-inflammatory cytokines recruit activated immune and
278 inflammatory cells to the sites of lesions, thus increasing and maintaining the inflammatory
279 condition [39]. *Hedyotis diffusa* is a slender, annual plant that widely spread in the Asian
280 country, which has been used to treat inflammation, and urethral infection [40]. And it could
281 protect the renal damage by down-regulating the levels of TNF- α , IL-1 β , IL-6, and
282 up-regulating the level of IL-10[41]. *Epimedium brevicornu* Maxim also shown an
283 anti-inflammatory effect in LPS-induced peritonitis by inhibiting the production of TNF- α ,
284 IL-1 β , and IL-6 [17]. Icariin is a major bioactive monomer, and studies have shown its
285 potential effect to treat immune and inflammatory diseases [42]. Berberine is an alkaloid
286 derivative extracted from a variety of plants, and Liu X' experiment reveals its
287 immunomodulatory effects in an autoimmune myocarditis rat model [43]. All these herbal
288 products had a synergic anti-inflammation effect.

289 In particular, the TNF- α level was usually high in the expressed prostatic secretions (EPS)
290 from men with CP/CPPS [44]. TNF- α is mainly derived from monocytes and macrophages.
291 The study found CD4⁺ T cells and macrophages are key factors in the development of
292 CP/CPPS[45]. Increased inflammasome may be a possible mechanism of CP/CPPS, and
293 inhibiting the inflammasome-related pathway may be a new therapeutic approach [46]. Some
294 studies revealed that inflammasome might be a target for pain therapy [47]. The essential
295 problem of CP/CPPS is congestion, collateral blockage, and inflammatory cells infiltration.
296 All these herbals play a vital role in reducing inflammation and activate blood circulation of
297 chronic diseases.

298 Our results also showed the therapeutic role of QTD in ameliorating urinary tract symptoms,
299 relieve pain, and reduce inflammation in CP/CPPS therapies. H&E staining of prostate tissue
300 has demonstrated that the high-dose group of QTD had a most significant anti-inflammatory
301 effect on CP/CPPS. TNF- α is a well-known pro-inflammatory cytokine. In the CP/CPPS
302 model group, the expression levels of TNF- α were higher than the control group. After
303 treatment, the TNF- α levels decreased significantly and were similar to the control group,
304 which demonstrated that QTD plays an important role in the immunomodulatory of the
305 CP/CPPS mice model. Here, we used Chinese medicines to treatment CP/CPPS, and its
306 possible mechanism of action is to suppress the immune response by reducing the release of
307 TNF- α .

308 CP/CPPS prostate involvement pain may be related to the axon reflex of the dorsal root
309 ganglion and cause neurogenic inflammation. TNF- α is involved in pain perception during
310 antigen-induced neurogenic inflammation [48]. Increased levels of TNF- α may also cause
311 neuronal damage. By upregulating the voltage-gated channels of dorsal root ganglion neurons,
312 uninjured neurons are involved in neuropathic pain [49]. The pain threshold was significantly
313 lower in the model group, which may be local cytokine production or local
314 neuroinflammation caused by autoimmunity. After 4 weeks of the intervention of QTD, the
315 pain in the mice was significantly relieved, this may be related to the level of cytokine TNF- α .
316 We only analyzed serum levels of TNF- α , which may be the principal limitation of this study.
317 In subsequent research, we will analyze more cytokines in the serum and prostatic tissue.

318 The current treatment for CP/CPPS is mainly using a combination of antibiotics, α -blockers,
319 and anti-inflammatory drugs. The improvement in symptoms and response to treatment is

320 very variable; for CP/CPSP, management will likely progress toward symptom-specific
321 rather than a generic, 'one strategy fits all' treatment [50]. However, the TCM formula has
322 multiple ingredients; it is a more comprehensive treatment for multiple symptomatic diseases.
323 Based on the UPOINT system [51], the QTD has unique advantages in urinary symptoms,
324 organ function, tenderness of muscles, and regulate psychology.

325 In this study, we revealed that Qianlie Tongli's decoction could reduce the number of urine
326 spots, improve the pain threshold, and reduce inflammatory lesion and inflammatory cells,
327 and decreases the TNF- α level in the serum. However, we additionally expect that Qianlie
328 Tongli's decoction may be useful for all kinds of prostatitis diseases.

329 **5. Conclusion**

330 CP/CPSP is an enigma for many researchers. Although its treatment is not yet absolutely
331 understood, traditional Chinese medicines have a significant effect on the medication for
332 CP/CPSP. This Chinese medicine formula (QTD) provides a new therapeutic approach to
333 CP/CPSP. However, to conclude the long-term effectiveness of this treatment, more clinical
334 study is needed to explore the practice of traditional Chinese medicine in the treatment of
335 CP/CPSP.

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341 **Conflict of interest**

342 All authors declare no conflict of interest.

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