Protective potential of the methanol extract of *Macrothelypteris oligophlebia* rhizomes for chronic non-bacterial prostatitis in rats

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Abstract: The protective potential of the methanol extract of *Macrothelypteris oligophlebia* rhizomes (MMO) for chronic non-bacterial prostatitis (CNP) in rats was investigated in the present study. Carrageenan-induced CNP in rats was established. Fifty rats were randomly divided into sham-operated (sham-ope) group, model group, positive control group (Cernilton at a dose of 148mg/kg body weight) and two MMO-treated groups (MMO at doses of 600mg/kg and 300 mg/kg body weight). The anti-prostatitis effect was evaluated by prostate index, the levels of interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-α), cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2), and histopathological examination. After 20 days of administration, MMO could significantly decrease prostate index and the levels of IL-10, TNF-α, COX-2 and PGE2 in serum and could improve the prostate morphology in comparison with the model group. In summary, these results suggest that MMO possesses protective effects on prostate, which might be beneficial to further development for the treatment of CNP.

Keywords: *Macrothelypteris oligophleba*, Methanol extract, Chronic non-bacterial prostatitis.

INTRODUCTION

Many men aged older than 50 years have been suffered from prostatitis, which is the third most common urologic diagnosis beside benign prostatic hyperplasia (BPH) and prostate cancer (Touma and Nickel, 2011). Chronic non-bacterial prostatitis (CNP) in the National Institutes of Health (NIH) classification belongs to type IIIa of prostatitis, which is characterized by chronic idiopathic pelvi-perineal pain and an inflammatory subtype with leukocytes expressed in their prostatic secretions, postprostate massage urine, or semen (Krieger *et al.*, 1999). It is a multifactorial problem and accounts for the majority of prostatitis case encountered in the community, which costs approximately $84 million annually (Duloy *et al.*, 2007). In North America, Europe, and Asia, CNP has been found to be highly prevalent among unselected men (Ku *et al.*, 2005). Above phenomena clearly indicate that CNP has become a considerable problem in international health care.

However, no standard therapies are confirmed to be available and effective for the treatment of male chronic pelvic pain syndrome (CNP and/or prostatodynia). Many forms have been implemented, with results that are often presented as unsatisfactory and unfortunate. Based on a concept of prostatitis, traditional treatment for chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) has been consequently centered on infection and inflammation of prostate (Nickel, 2008). No wonder the most common therapeutic methods are muscle relaxants, some antibiotics and anti-inflammatories such as terazosin (Cheah *et al.*, 2003), levofloxacin (Nickel *et al.*, 2003), or their combination therapy. Since the very small and single-center study showed no obvious benefits, unsupervised herbal-based therapies have gradually been acceptable and popular with patients in CP/CPPS (Nickel, 2008). The plant extracts like Cernilton (a pollen extract) and some bioflavonoids like quercetin have been devoted more and more attention and show better efficacy, because they have been reported to possess anti-inflammatory property and to have symptomatic relief in patients with chronic prostatitis syndrome (CPS) or BPH (Elist, 2006). Plentiful crude herbal water or ethanol extracts, because of the high concentration of flavonoids, have been studied to relieve inflammation and pain, such as *Serenoa serrulata*, *Epilobium parviflorum* and so on (Steenkamp *et al.*, 2006). Therefore, seeking various phyto-therapeutic agents with higher efficacy and lower side effect becomes rewarding, which is the intention of this study.

Macrothelypteris oligophlebia (Thelypteridaceae) is diffusely distributed in southwestern China. Its rhizomes have been used as a folk medicine mainly treating diseases such as edema, boils, burns, and roundworms (Xing, 2004). In the previous study the chemical constituents of this species have been identified mainly for flavonoids, including naringenin-4′-O-glucoside, 5,7-dihydroxy-6, 8-dimethyl flavanone, kaempferol-3-O-β-rutinoside, protoapigenin-4′-O-β-d-glucoside and so on, while the nephroprotective activity of *M. oligophlebia* was studied (Wu *et al.,* 2012). Besides, in the presence of

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some flavonoids compounds possessing strong anti-inflammatory effect, we designed this experiment to investigate the protective potential of the methanol extract of *Macrothelypteris oligophlebia* rhizomes (MMO) for CNP.

MATERIALS AND METHODS

Chemicals

Cernilton (containing pollen extract P5 70mg and EA10 4mg per tablet) was purchased from Nanjing Meirui Pharmaceutical Co. Ltd. Lambda-Carrageen an was provided by Sigma-Aldrich (St. Louis, Mo, USA). Enzyme-linked immunosorbent assay (ELISA) kits were supplied by R&D Systems Inc.

Plant material

M. oligophlebia rhizomes were gathered from Jiujiang city, Jiangxi province, China and identified by Prof. Ceming Tan, Forest Plants Specimen Mansion. A voucher specimen (MTF0812) was deposited in our department.

Preparation of methanol extract

The rough powder of air-dried *M. oligophlebia* rhizomes (1. kg) was obtained by using a mixer grinding at a high speed for 2 min. Then it was extracted with methanol at 75°C under reflux for 1h and the process was repeated 3 times. By using a rotary evaporator, all the filtered extracting solution was evaporated to eliminate as much solvent as possible under reduced pressure. Finally, the desirable extracts were desiccated in the vacuum dryer at 56°C for about 48h and the dried extracts (211.91g) were obtained.

Determination of total flavonoids content

Total flavonoids content of the extracts was estimated and expressed as rutin equivalent. Refer to similar experimental procedure (Xu *et al*., 2008), the content of total flavoniods was calculated by drawing standard curve.

Carrageenan-induced chronic non-bacterial prostatitis model

The whole experiments were performed in accordance with the European Community guidelines for the use and care of laboratory animals and approved by the Huazhong University of Science and Technology Committee on Animal Care and Use. Male adult Sprague-Dawley (SD) rats with a range of 180-220g weight were purchased from the Animal Research Center of Tongji Medical Center, Huazhong University of Science and Technology (Animal license number: SCXK (e) 2004-0007). They were housed at a controlled room with a 12 h light: 12h dark cycle under temperature 24±2°C and humidity 55±10%. They had access to standard diet and water ad libitum before they were used and during the entire experiment period. In the case of oral administration, rats were fasted for 12 h before testing.

For animal test, an appropriate weight of MMO was suspended in 600 or 300mg/kg body weight with distilled water. As positive control, Cernilton pollen extract was also dissolved with distilled water to get a dosage of 148 mg/kg here.

SD rat models of carrageenan-induced CNP (Radhakrishnan and Nallu, 2009) were used to evaluate the protective potential of MMO. Fifty SD rats were randomly divided into sham-operated (sham-ope) group, model group, positive control group and two MMO-treated groups (MMO 600mg/kg and MMO 300mg/kg) with animal number of 8, 12, 10, 10 and 10. In groups of model, positive control and MMO-treated, rats were treated with injection of 0.1ml 1% carrageenan (dissolved in saline) into the right and left lateral prostate under chloral hydrate (350mg/kg of body weight) anesthesia. The same operation was implemented with injection of 0.1ml physiological saline instead in the sham-ope group. Seven days after the operation, rats were kept for oral administration of 600 or 300mg/kg MMO in two MMO-treated groups and 148mg/kg Cernilton in the positive control group for 20 days. In the sham-ope group and model group rats were given saline with the same method for the same time.

Serum and tissue samples

After 20 days, the rats in every group were weighed and decapitated 24h after final administration. Blood was collected and sampled from the orbits, and serum sample was separated by centrifugation at 3000 r/min for 10min. Levels of IL-10, TNF-α, COX-2 and PGE2 in serum were analyzed using specific ELISA kits. The whole prostate was extirpated and weighed to calculate prostate index, which was the ratio of prostate weight to body weight (mg/kg). The right prostatic lateral lobes were rapidly excised and fixed in 10% neutral buffered formalin for 3 days, then embedded in paraffin, serially sectioned, and stained with haematoxylin-eosin. Histological analysis was performed on deparaffinised sections for pathological examination.

STATISTICAL ANALYSIS

Data were represented as mean ± SD values. Results were statistically analyzed by utilizing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistical significant at $p<0.05$.

RESULTS

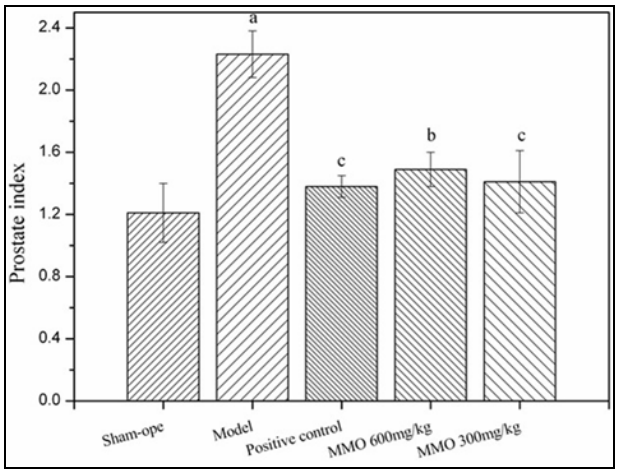
Total flavonoids content

The total flavonoids content in MMO was expressed in milligrams per gram of extracts. The MMO was found to contain 196.9mg/g total flavonoids.

Prostate index changes

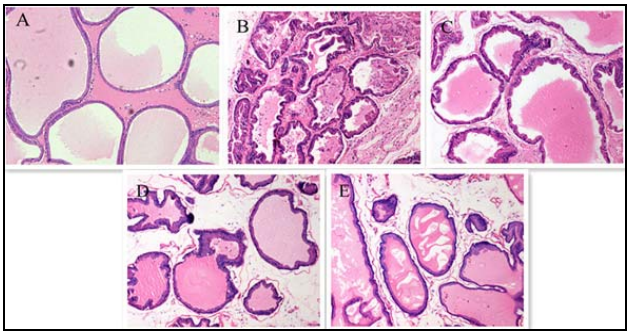
As shown in fig. 1, absolute prostate index in the model

group was significantly ($p<0.01$) higher than that in the sham-ope group, which elucidated that the carrageenan successfully induced CNP in the SD rats. Compared with the model group, the index in groups of positive control and drug treatment (MMO 600mg/kg and MMO 300mg/kg) were distinctly ($p<0.05$ or $p<0.01$) inhibited.



[a]$p<0.01$ compared with sham-ope group; [b] $p<0.05$, [c] $p<0.01$ compared with model group.

Fig. 1: Effect of MMO on prostate index in rats with chronic non-bacterial prostatitis



(A) sham-ope group, (B) model group, (C) positive control group, (D) MMO group (600mg/kg) and (E) MMO group (300mg/kg). Hematoxylin and eosin stain. Magnification × 100.

Fig. 2: Effect of MMO on histopathological changes in carrageenan-induced chronic non-bacterial prostatitis.

Histopathological examination

In the fig. 2, histopathological examination showed no obvious changes in the morphological structure of the prostate gland of rats in the sham-ope group. However, in the model group there was significantly different in morphological characteristic of the prostate gland, including irregular glandular shape and severe diffuse chronic inflammation characterised by numerous neutrophils, lymphocytes and other inflammatory cell infiltration, interstitial fibrosis and mild glandular hyperplasia. The Cernilton and MMO treatments remarkably ameliorated these changes in comparison with the model group.

ELISA for IL-10, TNF-α, COX-2 and PGE2

From the data in table 1, the concentrations of all four indicators had remarkably ($p<0.05$ or $p<0.01$) increased in the model group compared with the sham-ope group. It was displayed that there was no significant difference among the sham-ope, positive control and drug treatment groups, especially between the positive control and MMO 300 groups. Furthermore, MMO at a dose of 300mg/kg could conspicuously ($p<0.05$ or $p<0.01$) inhibited IL-10, TNF-α, COX-2 and PGE2 production with similar effect as Cernilton at an appropriate dose of 148mg/kg.

DISSCUSSION

In the present study, the protective potential of MMO was assessed on the rat model of carrageenan-induced chronic non-bacterial prostatitis. Injection of carrageenan into an air ponch in rats or mice has been documented to induce an inflammatory response that can be quantified by the volume of exudate produced, the infiltration of cells, and the release of inflammatory mediators (Duarte *et al*., 2012). The CNP model in the study showed chronic inflammation and fibrosis in the prostatic lateral lobes, further proving that carrageenan successfully established prostatitis.

The study found that the total flavonoids content of MMO was determinated up to 196.9 mg/g total flavonoids. Since flavonoids were characteristic of high antioxidant and anti-inflammatory activity, the high amount of total flavonoids implied the extract possessing a high antioxidant and anti-inflammatory activity. Taken into consideration of the safety and efficacy of the extract, we designed high-low doses of 600 mg/kg and 300 mg/kg as the desirable dosage (Chen *et al.,* 2012**).** Similarly, on the basis of pre-existing reference dose as well as the clinical dose that Cernilton was used, we advanced the dose of 148mg/kg as daily administration dosage of the positive control group (Xu *et al*., 2008; Lu *et al.*, 2011). From above results shown, MMO was well tolerated by all rats during the entire experiment with fewer side effects. In the fig. 1 and fig. 2, the decreased prostate index and the improvement of histopathological examination in the treatment groups showed that MMO had potential protective effect on prostate because of the inflammatory suppression of CNP.

An imbalance between pro-inflammatory and anti-inflammatory cytokines has been implicated some correlation with pelvic pain (Pontari and Ruggieri, 2004). IL-10 and TNF-α is usually detectable in men with CPPS (Miller *et al.*, 2002; Nadler *et al.*, 2000). As an anti-inflammatory cytokine, IL-10, which could be produced by various cells including B and T cells, will relatively down-regulate the expression of pro-inflammatory cytokines such as TNF-α and IL-1β by complicated mechanisms (Papadakis and Targan, 2000). In the table 1,

Table 1: Effect of MMO on IL-10, TNF-α, COX-2 and PGE2 in serum

Group, dose (mg/kg)	N	IL-10 (pg/ml)	TNF-α(pg/ml)	COX-2 (pg/ml)	PGE2 (pg/ml)
Sham-ope (-)	8	2.98±0.79	21.76±0.97	11.92±1.52	21.57±2.70
Model (-)	12	4.41±0.98 [a]	32.59±3.45 [a]	16.65±1.20 [a]	32.36±1.81 [a]
Positive Control 148	10	3.41±0.30 [b]	22.63±1.99 [c]	12.10±2.01 [c]	22.27±2.49 [c]
MMO 600	10	3.78±0.91 [b]	23.67±1.97 [c]	14.05+2.77 [b]	27.58±1.66 [c]
MMO 300	10	3.48±0.72 [c]	23.00±1.35 [c]	12.43±1.45 [c]	24.45±2.88 [c]

Data were represented as mean ± SD values.
[a]$p<0.01$ compared with sham-ope group; [b]$p<0.05$, [c]$p<0.01$ compared with model group.
IL-10: interleukin-10; TNF-α: tumor necrosis factor alpha; COX-2: cyclooxygenase-2, and PGE2: prostaglandin E2.

IL-10 and TNF-α in treatment groups was significantly inhibited compared with that in the model group, which implied that MMO might generate some influence on Th1/Th2 developmental pathways. MMO was suspected to regulate immune response by inhibiting immune cells (Th1/Th2 cell) to protect prostate from inflammation.

Of course, there are also other cytokines to mediate prostatitis progressing. COX-2 is reported to play an important role in the induction of pain and inflammation (Khan *et al.*, 2007). The multiple interactions of PGE2 with PGE2 receptor subtypes will ultimately result in pain and vasodilation (Hahn *et al.*, 2000). Thus, in the experimental study the interrelation among COX-2, PGE2 and prostatitis was established, which was seldom studied before. As shown in the table 1, a homologous phenomenon about COX-2 and PGE2 emerged again. It might be the result of MMO inhibiting biosynthesis of COX-2 to block the generation of inflammatory prostaglandins for the anti-inflammatory and analgesia effect on CNP.

CONCLUSION

All experimental results showed that oral administration of the methanol extract of *M. oligophlebia* rhizomes for 20 days exhibited strong protective potential through different mechanisms on rats with chronic non-bacterial prostatitis for the first time. Maybe some component in MMO plays a dominant role during the entire treatment period. In contrast, it is also possible that different compounds could be responsible for the various activities observed. More attention is essential to be devoted for further studies on exploring the bioactive components or fractions of MMO possessing protective potential on rats with CNP and the definite mechanism of action.

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