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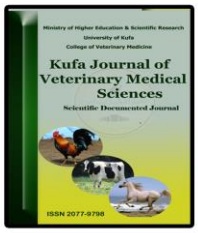

Effect of alcoholic extract of *urtica dioica* leaves and Aspirin in experimentally induced prostatitis in mice

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Abstract

Urtica dioica leaves were powdered and extracted with methanol 70 %. 17 beta-estradiol was used to induce prostatitis. Fifty mice were divided into five groups, The first control group (T1) , The second induction group (T2) was treated by distilled water, the third induction group (T3)was treated with crude extract of *Urtica dioica* leaves 300mg/kg B.W, the fourth induction group (T4) was treated with aspirin 50mg/kg B.W, the fifth induction group (T5)was treated with crude extract of *Urtica dioica* leaves 150mg/kg B.W and aspirin 25mg/kg B.W. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) for induction groups treated with 17 beta-estradiol showed significant decrease as compared with (T1), while the T3,T4 and T5 showed no significant difference as compared with T1. The result of testosterone hormone of (T3) showed significant increase as compared with (T1) and other groups. The results of body and prostate weight for T3,T4 and T5 revealed no significant difference as compared with T1. The fertility index of (T3) was increased as compared with T1 while the T4 was decreased as compared with T1. The histopathological changes after induction of prostatitis revealed hyperplasia of the prostatic glands and infiltration of inflammatory cells, while the T3 revealed the return of the acini to normal shape, and hydropic degeneration in the epithelium of prostatic glands disappeared. The T4 showed decreased hydropic degeneration, few inflammatory cell and epithelial hyperplasia, while the T5 was characterized by few inflammatory cell infiltration and the acini showed very decreased hydropic degeneration. In this study we concluded that the alcoholic *Urtica dioica* extract could markedly treat proststitis by its anti-inflammatory effect with reduce side effect of aspirin such as infertility.

Key words: prostatitis, *urtica dioica*, Aspirin and 17 beta-estradiol.

تأثير الخلاصة الكحولية لأوراق نبات القريص والاسبرين في التهاب البروستات المحدث تجربيا في الفئران

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الخلاصة:

تم استخلاص وتجفيف الخلاصة الكحولية لأوراق نبات القريص بأستخدام 70% كحول الميثانول . تم استخدام 17 بيتا استراديول لأستحداث مرض التهاب البروستات. قسمت الفئران(خمسين فأرة) إلى خمس مجموعات، المجموعة الاولى تركت كمجموعة سيطرة, المجموعة الثانية محدثة للمرض ومعالجة بالماء المقطر, المجموعة الثالثة محدثة للمرض ومعالجة بالخلاصة الكحولية لنبات القريص 300 ملغم/كغم من وزن الجسم , المجموعة الرابعة محدثة للمرض ومعالجة بالاسبرين 50 ملغم/كغم من وزن الجسم والمجموعة الخامسة محدثة للمرض ومعالجة بالخلاصة الكحولية 150 ملغم /كغم و الاسبرين 25 ملغم/كغم من وزن الجسم. اضهرت دراسة السوبر اوكساید دایمیوتیز والكلوتاثيون بيروكسديز للمجاميع المستحدثة والمعاملة ب 17 بيتا استرادول انخفاضا كبيرا بالمقارنة مع مجموعة السيطرة. وأظهرت المجموعة المعالجة بالخلاصة الكحولية زيادة كبيرة ل هرمون التستوستيرون بالمقارنة مع مجموعة السيطرة والمجاميع أخرى. كشفت نتائج وزن الجسم و البروستات للمجموعة المعالجة بالخلاصة الكحولية والمجموعة المعالجة بالاسبرين والمجموعة المعالجة بالخلاصة والاسبرين بعدم وجود فروقات معنوية كبيرة بالمقارنة مع مجموعة السيطرة. اضهرت المجموعة المعالجة بالخلاصة الكحولية ارتفاعا نسبيا ل دليل الخصوبة بالمقارنة مع مجموعة السيطرة في حين اضهرت المجموعة المعالجة بالاسبرين انخفاضا نسبيا لدليل الخصوبة مقارنتا بمجموعة السيطرة. اضهرت التغيرات التشريحية المرضية بعد تحريض التهاب البروستات تضخم في الغدد البروستات ونضوح الخلايا الالتهابية، في حين كشفت المجموعة المعالجة بالخلاصة الكحولية عودة عنيبات إلى الشكل الطبيعي، واختفاء التحلل الاستسقائي في ظهارة غدد البروستات. أظهرت المجموعة المعالجة بالاسبرين اانخفاضا للتحلل الاستسقائي، وعدد قليل الخلايا الالتهابية ، في حين اتسمت المجموعة المعالجة بالخلاصة الكحولية والاسبرين ب عدد قليل من الخلايا الالتهابية وانخفاض قوي للتحلل الاستسقائي. في هذه الدراسة نستنتج الى ان الخلاصة الكحولية لأوراق نبات القريص عالجت بشكل ملحوظ التهاب البروستات بواسطة تأثيرها المضاد للالتهابات مع تقليل الآثار الجانبية للأسبرين مثل العقم.

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Introduction:

Prostatitis is a condition that involves inflammation of the prostate and a common disease in males, the causes of prostatitis might be Uropathogens infection such as Escherichia coli, Enterococcus spp and Klebsiellaspp or non-infectious prostatitis such as hormonal imbalance, neurological dysfunction, a-adrenergic system abnormalities, urinary reflux into the, inappropriate cytokine release and autoimmune response (1). The symptoms of disease include pain during urination, difficulty in emptying the bladder completely or urinary frequency, pain in the testicles or other sites in the pelvic area, pain during or after ejaculation, small amounts of blood in the semen or even fever and chills with some acute cases of prostatitis (2).

Non-steroidal Anti-inflammatory medications such as aspirin drugs have been used for the treatment of prostatitis, but this group of drug has many side effects causing impairment of the later stages of spermatogenesis, gastrointestinal (GI) ulcerations,

nephrotoxicity, hepatotoxicity, breathing difficulty, nausea, weakness, dizziness, fatigue and autoimmune disorders (3). Although using frequent chemical drugs such as corticosteroids have been effective in reducing inflammation, side effects of these drugs are completely recognized and inevitable. Therefore, new studies introduces supplementary treatments, especially herbal medicine at low costs with minimum side effects (4).

Urtica dioica plant has been widely used by herbalists around the world for centuries is used for excessive menstrual bleeding, diarrhea, diabetes, urinary disorders and respiratory problems including allergies, arthritis pain, eczema, ulcers, asthma, diabetes, intestinal inflammation, prostate diseases for relief of benign prostatic hyperplasia and other prostate problems, antibacterial and febrifuge (reduces fever) with little or no side effect (4). This study aimed to reduce the side effect of non-steroidalanti-inflammatory drug (aspirin) by using crude extract of *Urtica dioica* and to compare between the effect of alcoholic extract and aspirin on prostatities.

Material and methods:

Extraction of *Utrica dioica* leaves: The leaves of *Urtica dioica* were powdered and extracted with methanol 70 % and placed on hot plate magnetic stirrer for 24 hours, and then was filtered using big and small leach papers. The extract was stored in incubator at temperature of in 50°C in order to evaporate the alcohol after that kept in deep freeze (5).

Preparation of stock solution from crude *Utrica dioica* leaves extract: (300) mg of the dried extract was dissolved in distilled water; volumes were completed to (10) ml. 0.1 ml of the stalk was given to each (10) gm B.W (6).

Experimentally Animals: albino Swiss mice weighting 30g were obtained from the animal house of Biotechnology central-Al-Nahrain University. Mice were housed plastic cage 30x10x10 cm placied in the room until the beginning of experiments. Standard rodent diet (commercial feed pellets) and Tap ad.lib. Water was freely available.

Induction of prostatitis: 17 beta-estradiol (E_2; 250 microgram/kg.) was injected subcutaneously into adult male mice for 30 days to induce prostatitis, serum Superoxide dismutase (SOD), serum glutathione peroxidase (GPx), and histological changes were also evaluated after 30 days from induction to insure from appear the disease (7).

Experimental designs: fifty mice were divided into five groups, the period of treatment in all groups was 30 days: The first (T1): control group without any treatment (negative control group). The second group induction (T2) was treated by distilled water given orally by stomach tube (positive control). The third group induction (T3) was treated by crude extract of *Urtica dioica* leaves 300mg/kg B.W treated orally the fourth group induction (T4) was treated by aspirin 50mg/kg B.W treated orally. The fifth group induction (T5) was treated by crude extract of *Urtica dioica* leaves 150mg/kg B.W and aspirin 25mg/kg B.W given orally.

Parameters used in these experiments:

Superoxide dismutase (SOD) and glutathione peroxides (GPx): Blood samples were collected and SOD

enzyme in serum was measured by SOD Assay Kit-WST (Dojindo), GPx enzyme in serum was measured by a GPx Assay Kit (Cayman).

Testosterone hormone: the animals were sacrificed and blood was collected by cardiac puncture and serum was separated. Concentration of testosterone was determined by Radio immunoassay(RIA) (8).

Fertility index: This index was determinate according to (9). In each stage, each male mouse was caged separately with one female of proven fertility in the evening for 6 days. Presence of sperms in the vaginal smears examined on the next day morning indicated that the females had mated to the particular males and the day of mating was taken to be days 1 of pregnancy. Fertility test was considered positive when implantation sites were present.

Body weight and prostate weight: The body weight of males was measured at first day before and after dosage for 30 days by using a balance. In addition the prostate weight was measured after treatment.

Histological study: all groups were taken the parts from prostate after killed it. These samples were taken for histological study and these were kept in 10% formalin solution until the time of sections (10).

Statistical analysis: the ready program SAS (24) was used in statistical analysis for study the effect of different treated in adjective studies and the significant between mean was compared with less significant LSD.

Results:

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes and Testosterone hormone: the results of serum SOD and GPx in group treated with 17 beta-estradiol (E_2; 250 microgram/kg) showed significant decrease ($P \leq 0.05$) as compared with control group (table 1).

Table (1): Effect of 17 beta-estradiol (E_2; 250 microgram/kg.) on superoxide dismutase (SOD) unit/ml, glutathione peroxidase (GPx) unit/ml after 30 days from induction.

Group / Parameters	T1	T2
SOD	33.10± 7.06 a	29.99 ± 5.04 b
GPx	1.095± 0.0010 a	0.620 ±0.071 b

*****Data taken as mean ± SE

***** Different capital letters mean significant difference (p< 0.05) between raw numbers.

Whereas the results of SOD and GPX enzymes in the T3,T4 and T5 groups showed no significant difference (P≤ 0.05) as compared with T1 group . The result of testosterone hormone of (T3) group showed significant increase (P≤ 0.05) as compared with (T1) and others group, while the T5 group showed return the testosterone hormone to normal level (table 2).

Table (2): Effect of alcoholic extract of *Urtica dioica*, aspirin, *Urtica dioica* extract with aspirin and distilled water on superoxide dismutase (SOD) unit/ml, glutathione peroxidase (GPx) unit/ml and testosterone hormone (ng/ml) after 30 days from treatment.

Group / Parameters	T1	T2	T3	T4	T5
SOD	33.10 ± 7.06 a	28.61± 3.62 b	33.09 ± 6.96 a	32.99 ± 7.20 a	32.81 ± 7.01 a
GPx	1.095± 0.0010 a	0.612 ± 0.071 b	1.130 ± 0.0022 a	1.019 ± 0.022 a	1.021 ± 0.052 a
Testosterone hormone	6.60 ± 0.089a	1.19 ± 0.022b	9.01 ± 0.050c	3.08 ± 0.015d	5.90± 0.031a

*****Data taken as mean ± SE

***** Different capital letters mean significant difference (p< 0.05) between raw numbers.

Body and prostate weight: The distilled water treated group (T2) showed significantly reduction (P≤ 0.05) body weight compared to the control group and other groups (T3,T4 and T5), while the and prostate weight showed significant increase (P≤ 0.05) as compared with control and other groups (T3,T4 and T5). However, no significant difference in body and prostate weight was noted in extract group(T3), aspirin treated group (T4) and extract with aspirin group (T5) as compared with control group (table 3).

Fertility index: the result of the fertility index in extract group (T3) was increased as compared with control group (T1) while the aspirin treated group (T4) was decreased as compared with control group (T1) (table 3).

Table (3): Effect of alcoholic extract of *Urtica dioica***,** aspirin, *Urtica dioica* extract with aspirin and distilled water on body weight (g), weight of prostate (mg) and fertility index (%) of mice after 30 days of treatment.

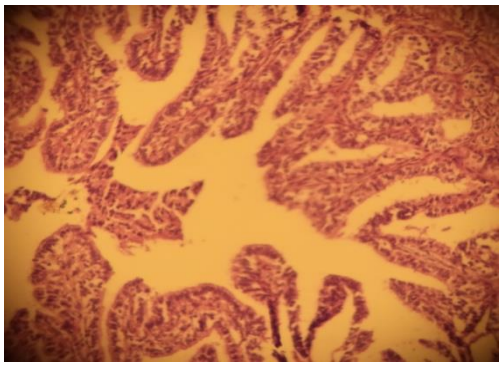
Group / Parameters	T1	T2	T3	T4	T5
Body weight	32.40 ± 1.67a	28.10± 1.01b	31.65± 1.70a	32.02± 1.55a	31.99± 1.40a
Weight of prostate	40.93± 0.050 a	44.11 ± 0.011 b	40.52 ± 0.031 a	40.22 ± 0.027 a	40.31 ± 0.082 a
Fertility index	91%	30%	95%	42%	85%

*****Data taken as mean ± SE

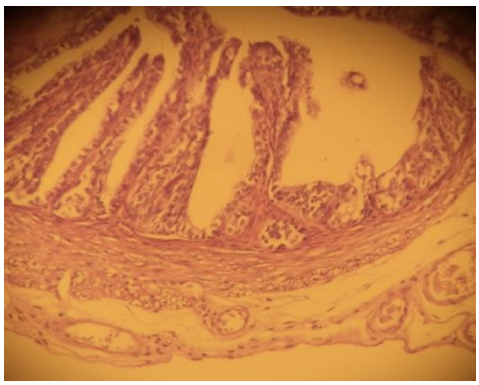
***** Different capital letters mean significant difference ($p< 0.05$) between raw numbers.

Histopathological changes:

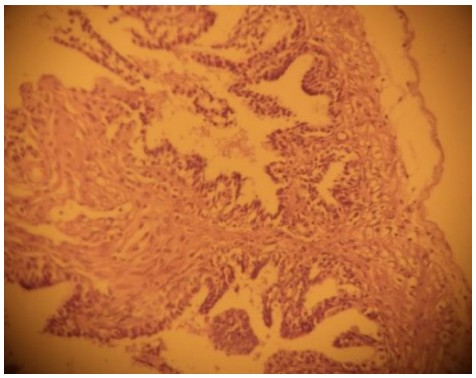
After 30 days of prostatitis induction with 17 beta-estradiol and after treatment by distilled water for 30 days revealed hyperplasia and proliferation in the epithelium of the prostatic glands and infiltration of inflammatory cells as copared with control group(figure1, 2), in(figure 3) group treated with *Urtica dioica* extract for 30 days noticed return the acini to normal shape, and disappearance of hydropic degeneration in the epithelium lining of the acini of prostatic glands. The group treated with aspirin for 30 days showed decrease of hydropic degeneration, few inflammatory cell and epithelial hyperplasia as compared with control group (figure 4), while the group treated with *Urtica dioica* extract with aspirin for 30 days was characterized by few inflammatory cell infiltration and the acini showed very decrease hydropic degeneration as compared with control group (figure 5).



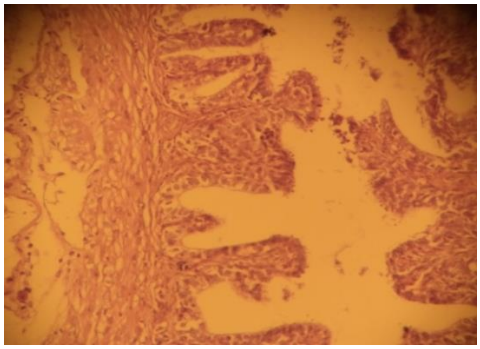
Figure(1):Histopathological section in prostate of mouse treated with 17 beta-estradiol for 30 days showed hyperplasia and proliferation in the epithelium of the prostatic glands giving papillary projection and there is sloughing and infiltration of inflammatory cells (H&E. X400).



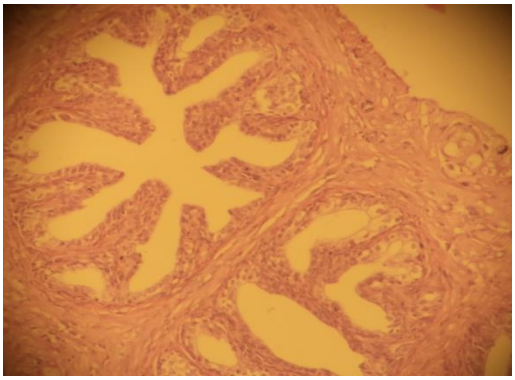
Figure(2):Histopathological section in prostate induction of mouse treated with distilled water for 30 days showed hyperplasia and proliferation in the epithelium of the prostatic glands and infiltration of inflammatory cells (H&E. X400).



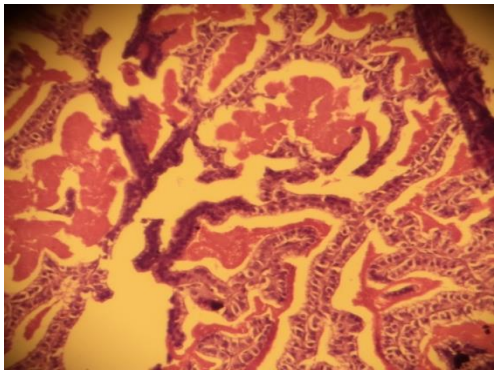
Figure(3): histopathological section in prostate induction of mouse treated with *Urtica dioica* extract for 30 days showed return the acini to normal shape, and disappear hydropic degeneration in the epithelium lining the acini of prostatic glands (H&E. X400).



Figure(4): histopathological section in prostate induction of mouse treated with Aspirin for 30 days showed decrease of hydropic degeneration, few inflammatory cell . (H&E. X400).



Figure(5): histopathological section in prostate induction of mouse treated with *Urtica dioica* extract with Aspirin for 30 days distinguished by few inflammatory cell infiltration and the acini showed very decrease hydropic degeneration (H&E. X400).



Figure(6) histological section in prostate of control mouse showed normal prostate tissue (H&E. X400).

Discussion:

Urtica dioica plant has been widely used to treat inflammatory disease and this herbal medicine is characterized by low cost with minimum side effects (4). So *Urtica dioica* extract was chosen to treat prostatitis.

The decrease in SOD and GPx enzymes in the 17 beta-estradiol induction group might be attributed to 17 beta-estradiol causing decrease in catalase activity and increase of lipid peroxidation lead to autoimmune prostatitis additionally decrease the activity of anti-oxidative enzymes refer to relation between oxidative stress and prostatitis, (11), while the extract of *Urtica dioica* return the SOD and GPx enzymes to normal level might attributed to potent antioxidant activity of *Urtica dioica* which contains **phenolic compounds**, especially flavonoids which is generally have antioxidant potential that scavenge free radicals, hydrogen peroxide and superoxide anion radicals and to chelate heavy metals that play important role in increase fertility (12) which reported that constituents from extract can regulate glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase in vivo, whereas the SOD and GPx enzymes result of aspirin treated group might attributed to improve antioxidant status by increasing plasma Nitric oxide (NOx) and Glutathione (GSH) by attenuating the induced oxidative stress, Asprin was presumably acting through its antioxidative properties, in particular, inhibition of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) -mediated peroxide production (13).

It is well recognized that mice treated with 17-b estradiol showed reduce body weight; this might be associated with the inhibition of growth hormone release in the pituitary gland by estrogen and a lowering of the serum insulin-like growth factor by estrogen (14). In addition the 17-b estradiol produces increase in prostate weight this might attributed to estrogens, influences on prostate tissue by stimulating prostate-cell growth and aromatase levels , also this result explained the testosterone levels decline (as higher aromatase levels convert testosterone to estrogen), and the prostate gland becomes enlarged, this results agreed with results reported by (15).

The results of *Urtica dioica* extract group indicated the return of prostate weight to normal weight could be attributed to the interfere or block a number of hormone-related chemical processes in the body that are implicated in the development of proststitis, in addition the extract has demonstrated the ability to stop the conversion of testosterone to dihydrotestosterone (by inhibiting an aromatase and 5-alpha reductase required for the conversion), as well as to directly bind to SHBG itself - thereby preventing SHBG from binding to other hormones, as well as it decrease the production of estrogens and increase testosterone hormone by inhibiting an enzyme required for their production lead to inhibit of prostatic growth factor interaction and inhibit of membrane sodium and potassium-adenosine triphosphate in the prostate, which results in the suppression of prostate cell metabolism and growth, this results agreed with results recorded by (16).

The result of body weight in *Urtica dioica* extract treated group extract revealed a potential utility of safer herbal medicines as an antistress agent as they can withstand stress without altering the physiological functions of the body, this might improve the weight of body , agreed with (17).

The results of body and prostate weight of aspirin treated group showed no significant difference as compared with control group this could be Aspirin acting as anti-inflammatory drug in treating disease of prostatitis by block cycloxygenase COX-2-related metabolism and inhibiting prostate cell growth, in addition it is considered non-androgenic in nature, since androgens are known to possess anabolic activities. Similar report was given by (18).

The result of decrease testosterone hormone level in aspirin treated group could indicate that aspirin inhibited the mechanism intervening in the process of hormone synthesis in the Leydig cells (19).

The decrease of fertility in distilled water treated group (T2) might be attributed to 17-b estradiol increased free radical and decreased total antioxidant level in semen in addition to decrease level of testosterone hormone lead to predict the possibility of infertility, suggesting that antioxidant supplements might be helpful for animals with prostatitis (20). The *Urtica dioica* extract increase fertility index suggested by increase antioxidant enzyme and increase testosterone hormone which is available for promoting male vitality and youthful sexual function (21).

The decrease of fertility index of aspirin treated group could be attributed to aspirin causes decrease in the activities of sorbitol dehydrogenase , hyaluronidase , decrease in the number of spermatids, decrease in testosterone hormone , in addition aspirin causes impairment of the later stages of spermatogenesis ,this result agreed with results reported by(22). The improvement in fertility index of extract with aspirin treated group could regarded to the extract causing increase testosterone hormone and the potential utility of herbal medicines as antistress agents have been reported as they can withstand stress without changing the physiological functions of the body that cause decrease side effect of aspirin (17).

The histopathological result of mice treated with 17-Bestradiol after 30 days might be attributed to 17-Bestradiol is partly related to the local aromatization of testosterone and inhibition of dopamine secretion at the hypothalamus, and dopamine deficiency enhances the production and secretion of prolactin that eventually causes inflammation of the prostate, as well as estrogen bound to sex hormone binding globulin SHBG, are usually carried to the receptor sites on the prostate gland and once there in excessive amounts, it can stimulate prostate tissue cells to divide and grow (23), the histopathological section of extract treated group showed return of the acini to normal shape better than aspirin treated group as compared with control group, indicated that *urtica dioica* have antiproliferative effect by directly inhibiting cell proliferation and blocking binding of epidermal growth factor to its receptor by inhibition adenosine deaminase activity of prostate tissue, this results agreed with (16) in addition the *Utrica dioica* extract has reduced the inflammation by inhibiting release of peripheral inflammatory intermediates Such as cytokines and *Tumor necrosis factor* (TNF) which are the most important inflammatory mediators Similar report was given by (24). The histopathological section of aspirin group attributed to aspirin acts directly on prostate epithelial cells to alter COX-2-related metabolism by reducing the synthesis of prostaglandins and inhibit prostate cell growth (25) whereas the histopathological section in extract with aspirin treated group showed very

decrease hydropic degeneration in acini as compared with aspirin and control groups , this referred to acceleratory effect of *Utrica dioica* extract in reduce inflammation (24) in addition to effect of aspirin drug by block COX-2 (25) .

Conclusion: This study showed that alcoholic *Urtica dioica* extract could markedly treat proststitis by its anti-inflammatory effect with reducing the side effect of aspirin such as infertility.

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