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Research Article

Effect of In-Vivo Chemical Cystitis on Citrate Synthase, Calcium Atpase, and Serca activities in Rabbits Treated with Jack Bean Supplements

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Abstract

Introduction

The high prevalence of cystitis associated with Urinary Tract Infections and other conditions such as non-bacterial cystitis and interstitial cystitis warrants further investigation into its etiology of causing bladder dysfunction. A model of cystitis in the rabbit urinary bladder was used to analyze how cellular processes may lead to the decreased compliance and contractility frequently associated with this condition. JB (Canavalia ensiformis) is a commonly used natural supplement in Chinese medicine to help treat the symptoms of this condition. JB (JB) is thought to confer bladder protection through its capacity as an antioxidant, thereby mitigating oxidative stress and preventing harmful cellular changes.

Methods

In order to obtain a more precise picture of how cystitis leads to bladder dysfunction and how antioxidants may offer protection, Citrate Synthase and SERCA assays were conducted in order to observe possible changes in mitochondrial function and calcium regulation.

Results

Previous study demonstrated that chemical cystitis impaired bladder contractility and compliance, but oral administration of JB demonstrated a significant beneficial effect by protecting bladder function. In the current study, significant decreases were observed in Citrate Synthase and SERCA activities following cystitis in the mucosa, suggesting damaged mitochondria and decreased ATP production, whereas no changes were seen in Calcium ATPase activity.

Discussion

This suggested the greater sensitivity of mitochondria and sarcoplasmic reticulum (SR) to oxidative stress compared to transmembrane calcium pumps. The bladder protection conferred by JB was likely mediated in part through its antioxidant capacity and preservation of mitochondria and SR ultimately support its continued use as a natural supplement to assist in the treatment of cystitis.

Introduction

Inflammation of the bladder, known as cystitis, is a common medical condition frequently associated with urinary tract infections (UTIs) [1-3]. UTIs affect 40-60% of women during their lifetime, which occurs four times more frequently in women than men because of anatomical differences. Other causes of cystitis include over distension, interstitial cystitis, and non-bacterial cystitis. All forms of cystitis are associated with increased frequency and urgency of urination, decreased volume

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at micturition, abdominal pain and sometimes painful urination. All forms of cystitis are related to damage of the bladder urothelial layer, which increases permeability, inflammation, and increased bacterial adherence to the mucosa [3,4]. Oxidative stress is also thought to play a role by damaging various cellular membranes through the generation of harmful free radicals.

The rabbit urinary bladder has proven to be a useful and versatile model because of its similarity to the human bladder in regards to frequency of urination, and similar contractile and micturition mechanisms [5-8]. In this study, a model of cystitis was induced by intravesical administration with protamine sulfate and uric acid [6,9]. The specific aim of these studies were to determine the mechanism by which the Chinese plant product Jack Bean(JB) [10] was used successfully in preventing both urological and contractile dysfunctions following the induction of chemical cystitis.

Canavalia ensiformis: common name Jack Bean (JB)is a legume which is used for human nutrition. C. ensiformis is a twining plant up to 1 meter (3.3 ft) in height. It has deep roots which makes it drought resistant and can spread via long runners. The flowers are pink-purple in colorand the pods can be up to 36 centimeters (14 in) in length with large white seeds. The young foliage is also edible (Wikipedia).

Two of the major components of the JB are urease and concanavalin A. Concanavalin A (ConA) is a lectin (carbohydrate-binding protein) and member of the legume lectin family [11-14]. It binds specifically to certain structures found in various sugars, glycoproteins, and glycolipids. ConA is a plant nitrogen which is known for its ability to stimulate mouse T-cells. It has also been found to be effective in the treatment of liver cancer and other tumors. Ureases, functionally, belong to the superfamily of amidohydrolases and phosphotriesterases. It is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia [11-14]. These toxic properties have been completely eliminated by a proprietary methodology.

JB is also highly regarded for its protective antioxidant effects in various disease states [15,16]. It has a long history of use in Chinese medicine as a natural supplement for various ailments [17]. More specifically, it is also used to help treat the symptoms of cystitis. As such, this compound was an ideal candidate to use in our rabbit cystitis model [10]. The results of our initial study was that oral JB preparation significantly protected the bladder from the dysfunctional effects of protamine sulfate – uric acid on bladder function including significantly decreased bladder compliance and contractile responses [10].

The current study was designed to determine the possible underlying biochemical mechanisms. Citrate Synthase, Calcium ATPase, and Sarco/endoplasmic reticulum Calcium ATPase (SERCA) assays were performed to observe the changes in these enzymes [18,19]. Citrate Synthase is a biomarker for mitochondrial function because this enzyme is the first step in the Krebs Cycle. SERCA is a biomarker for sarcoplasmic reticular (SR) calcium storage and release function. Calcium ATPase of the plasma membrane provides energy for the movement of Ca2+ out of the cell.

As mentioned above, it was found that both contractility and compliance were decreased by the cystitis, and that JB provided significant protection. Correlating these changes with Citrate Synthase and SERCA activity should allow for a better understanding of the etiology of cystitis in addition to observing how the protective effects of this natural supplement on bladder function are mediated.

Methods

All methods were approved by the IACUC and Research and Development Committees of the Stratton VA Medical Center, Albany, NY.

Twelve adult male NZW rabbits were divided into 3 groups of 4 rabbits each. Group 1 were control rabbits that did not receive cystitis. Each rabbit received cystometry before beginning the study and at 2 and 4 weeks, the end of the study. Group 2 were rabbits that underwent cystitis but did not receive JB supplementation. They also underwent cystometry prior to entering the study and at 2 and 4 weeks. Group 3 were rabbits given a suspension of the JB preparation (100 mg/ml) at 1 ml/kg by gavage daily for 2 weeks prior to inducing cystitis and for 2 weeks following cystitis. Each rabbit received cystometry prior to entering into the study, immediately prior to inducing the cystitis, and at 1 and 2 weeks post cystitis, the end of the study.

For Groups 2 and 3, the cystitis was induced as follows. Immediately following cystometry, each rabbit was sedated with ketamine/xylazine (25 mg/10 mg, im). Under sterile conditions, the urinary bladder was catheterized with an 8 Fr. Foley catheter, emptied, and then filled with 25 ml of a saline solution containing protamine sulfate (10 mg/ml) + uric acid (100 mg/ml). The solution remained in the bladder for 30 minutes, and the bladder was then drained and washed three times with 50 ml of saline. The rabbits were allowed to recover for two weeks. After the recovery period, the bladders were excised and the tissue was separated into muscle and mucosa. These tissues were stored at -80°C for biochemical analyses.

The instrument used for both assays was a Hitachi U-2001 Spectrophotometer. For the Citrate Synthase Assay[18-20], tissues were homogenized at 50 mg/mL in a pH 7.5 Tris Buffer. These samples were then spun in a centrifuge at 1,500 g for 10 minutes, and the

supernatant was saved and the pellet discarded. 1mM DTNB was prepared in a pH 8.1 Tris-HCl buffer. The 10mM oxaloacetate was prepared in deionized water and then buffered with diluted bases and acids to a pH of 7.6. In a test tube, 900µL of sample was then mixed with 100µL of Triton X and vortexed thoroughly. In a quartz cuvette, 40µL of this Triton X and sample preparation was added to 1.1 mL of the Tris pH 7.5 buffer, in addition to 100µls 1mM DTNB and 30µls of 24.6mM acetyl CoA. The cuvette was then placed in the spectrophotometer and read for a scan time of 200 seconds and a cycle time of 60 seconds. A setup was used to ensure adequate stirring, using stir bars, and heat, using a 40°C warm water bath. After this initial reading, 50µL of 10mM oxaloacetate was added to each cuvette while they were still in the spectrophotometer and a second reading was done with a 1000 second scan time and a 120 second cycle time. All samples were run in duplicate, and a control was performed daily utilizing everything except the sample. The data collected was analyzed using Microsoft Excel and Sigmaplot to produce the final figures and Sigmastat for statistics using one way ANOVA and the Tukey test for individual differences.

For the calcium ATPase and SERCA assay [18-20], the previously prepared 50 mg/mL samples from the Citrate Synthase assay were diluted 1:5 in the pH 7.5 Tris buffer to obtain a sample with a concentration of 10 mg/mL. Thapsigargin at a concentration of 1mM was used from premade aliquots in which they had been dissolved in DMSO. Similarly, aliquots of 20mM ATP were premade in the pH 7.5 Tris buffer. Both the thapsigargin and ATP were made in the beginning of the experiment and stored at 20°C. A solution of 12.5% trichloroacetic acid (TCA) was made in deionized water. A solution of 10% ammonium molybdate was prepared in 10N sulfuric acid (H_2SO_4). For the standard curve, a solution of 2mM monopotassium phosphate (KH_2PO_4) was prepared in the pH 7.5 Tris buffer. Made daily was a solution of 0.18M ferrous sulfate molybdate in 1% acidified ammonium molybdate by dissolving 1.0g of ferrous sulfate ($FeSO_4$) in 16mL of deionized water and 2mL of the ammonium molybdate to obtain a final volume of 20mL. The samples were run in duplicates and divided into three different setups, one with thapsigargin and ATP, one without ATP, and one with ATP but without thapsigargin. A control was also performed with everything except the sample and without thapsigargin. All of the setups had 375µL of sample added (375µL of Tris buffer for the control), and then 50µL of both $CaCl_2$ and EDTA. Into only the thapsigargin group, 5µL of thapsigargin was added. All groups besides the non-ATP group then received 25µL of ATP (the non-ATP group received 25µL of Tris buffer instead). This amounted to a total volume of 500µL for all groups. The sample groups, but not the control group, were then incubated in a warm water bath at 37°C for 40 minutes. A standard curve was then prepared using the KH_2PO_4 and the Tries buffer for dilutions to obtain a final curve with concentrations of 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0mM. Once the samples were removed from the water bath, 500µL of the 12.5% TCA was added to all tubes, including the standard curve and the controls. Subsequently, 500µL of 0.18M ferrous sulfate molybdate was added to all tubes, giving a final volume of 1.5mL. The sample tubes were then spun for 2 minutes at 27,000 g. The curettes were then read in the spectrophotometer. All data was analyzed graphed and statistics performed as described above. Calcium ATPase activity was calculated from the ATP - thapsigarginsamples; SERCA activity was obtained by calculating the difference between the ATP + thapsigargin and ATP - thapsigargin.

Results

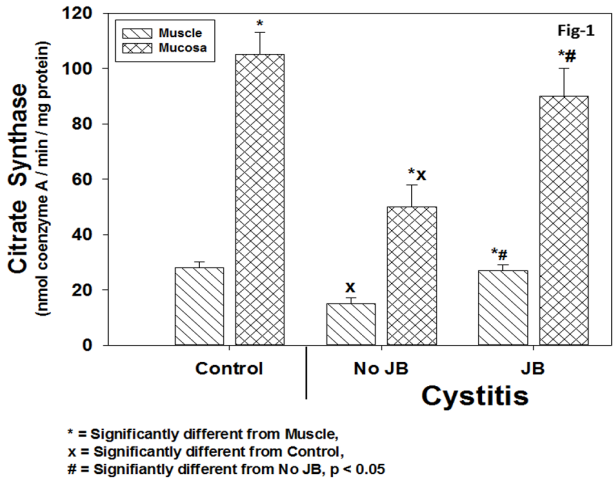

Figure 1.

Figure 1 shows the citrate synthase activity for the three groups. As observed previously, the citrate synthase activity of the mucosa was significantly higher than the muscle for all three groups. Both muscle and mucosa activities of the cystitis no JB group were significantly lower than either the control. Pre-treatment with JB resulted in cystitis having no effect on the CS activities of both the muscle and mucosa.

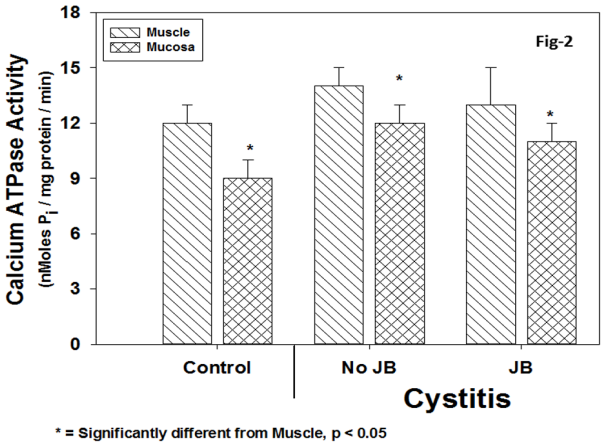

Figure 2.

Figure 2 displays the total calcium ATPase activity for the three groups. For all three groups, the mucosa had a small but significantly lower activity than the muscle. Cystitis had no effect on either the calcium ATPase activity of the muscle or mucosa, and there were no effects of JB on either muscle or mucosa.

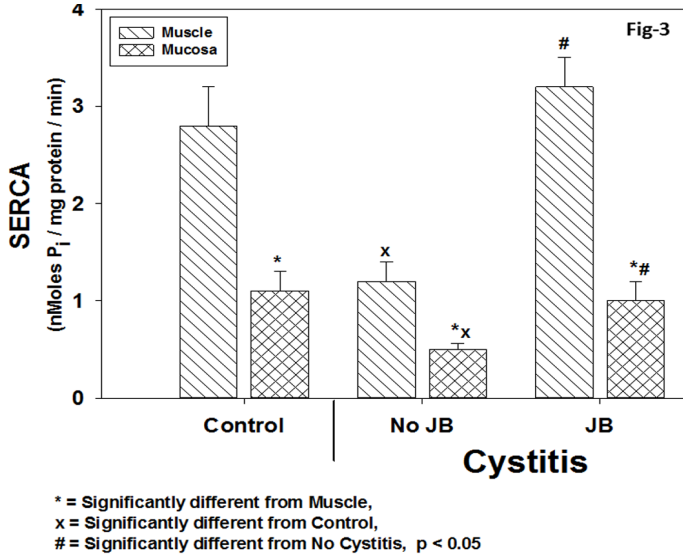

Figure 3.

Figure 3 displays the SERCA activity for the three groups. The SERCA activity of the mucosa was significantly lower than the muscle for all three groups. Both muscle and mucosa SERCA activities of the cystitis no JB group were significantly lower than the control group. Pre-treatment with JB resulted in cystitis having no effect on the SERCA activities of both the muscle and mucosa.

Discussion

As mentioned in the introduction, in a previous study, the chemical cystitis model was found to be effective in mediating changes in bladder function, resulting in decreased compliance, decreased volume at micturition, and decreased contractility[10]. These changes are consistent with the response of humans to cystitis[21].

Citrate synthase is the first step in the metabolism of glucose in the mitochondria, and it is an excellent bio-marker for mitochondrial function [19,22]. Dramatic differences were seen between how cystitis altered the activity of Citrate Synthase compared to Calcium ATPase. As observed previously, citrate synthase activity was significantly higher in the mucosa than the muscle [23-25], whereas the opposite was true for the total Calcium ATPase and SERCA activities.

The mucosa has a significantly higher metabolic rate than the muscle [24,26]. Mitochondrial enzyme activities such as citrate synthase and cytochrome oxidase are significantly higher in the mucosa than in the muscle [24,26], as is glucose metabolism [27]. However, the concentration of high energy phosphates (ATP and Creatine Phosphate) are significantly lower in the mucosa compared to muscle, as is the activity of the important enzyme creatine phospho-kinase (the enzyme that transfers a phosphate from creatine phosphate to ADP thus maintaining ATP concentrations high) is significantly lower in the mucosa than in the muscle [24,26]. The result is that the mucosa is significantly more sensitive to anoxia and ischemia than is the smooth muscle [24,26].

The higher citrate synthase (mitochondrial) activity in the mucosa is related to the localization and density of the mitochondria in the mucosa. The data from the Citrate Synthase shows dramatic decreases in activity induced by the cystitis in the muscle and mucosa. The CS activity of the cystitis +JB group was significantly higher than the cystitis – JB group, and was not significantly different from control both for the muscle and mucosa. It is likely that the preservation of mitochondrial function by JB was mediated by acting as an antioxidant and limiting free radical damage to the mucosal and muscle mitochondrial membranes.

The mitochondria are critical because of their production of ATP, which is then used to stimulate contraction through its activation of muscarinic receptors and calcium release. ATP is also what powers the Calcium ATPase pumps on the sarcoplasmic reticulum (SR) and the cellular membrane. The relationship between the roles of ATP and calcium is both complex and intimate, and in this case the severe decline in mitochondrial function suggests how susceptible it was to the oxidative stress induced by the cystitis. The subsequent protection of mitochondrial function by JB appears to be one of the key ways that antioxidants protect cellular function.

Cystitis significantly reduced contractile responses to all forms of stimulation. This is consistent with both the decreased CS and SERCA activities of the bladder muscle and mucosa. One of the most probable reasons is that the mucosal citrate synthase activity decreased to a greater extent than the decreased CS activity of the muscle relates directly with the higher metabolic activity [24,26]. of the mucosa, and greater sensitivity to anoxia and ischemia [23-25]. Decreased mucosal metabolism would lead directly with damage to the permeability barrier function of the mucosa. This would allow caustic and damaging substances through the mucosa and into the smooth muscle compartment. The result would be significant damage to the smooth muscle directly thus affecting the contractile responses to all forms of stimulation.

The decreased SERCA activity mediated by cystitis would also directly affect contraction based on the effects on SR calcium storage and release.

The oxidative stress associated with the cystitis appears to have been far more damaging to the metabolic activity of the mucosa than to the muscle. This is not necessarily surprising because of the higher dependence of the mitochondria on oxygen and other molecules relative to the mechanistically simpler enzyme pumps. It would be interesting to evaluate calcium-activated proteases and lipases such as calpain and phospholipase A2 which are activated only under high intracellular free calcium conditions [28-30].

In summary, it was found that JB confers a protective advantage on the bladder in a model of in-vivo cystitis. One aspect appears to have been through the protection of the SR and mitochondrial functions in the mucosa and muscle. These findings certainly shed some light on the biochemical changes induced by cystitis and will hopefully encourage further investigation into how cystitis impairs bladder function and ways to mitigate this process. The clinical significance of this condition due to the frequency of urinary tract infections (UTIs) in the adult population, particularly females, certainly warrants a more complete understanding. Although the mechanisms are still being uncovered, the ability of JB to protect both contractile and biochemical responses in our cystitis model supports its continued use as a valuable natural supplement to alleviate symptoms of cystitis.

Conclusion

Mitochondria and SR display greater sensitivity to oxidative stress compared to transmembrane calcium pumps. Protection by JB was mediated in part through its antioxidant capacity. and preservation of These results support its continued use as a natural supplement to assist in the treatment of cystitis.

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References

1. McGrother CW, Donaldson MM, Hayward T, Matthews R, Dallosso HM, et al. (2006) Urinary storage symptoms and comorbidities: a prospective population cohort study in middle-aged and older women. Age Ageing 35(1): 16-24.

2. Foxman B (2014) Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am 28(1): 1-13.

3. Costantini E, Lazzeri M, Porena M (2013) GAGs and GAGs diseases: when pathophysiology supports the clinic. Urologia 80(3): 173-178.

4. Iacovelli V, Topazio L, Gaziev G, Bove P, Vespasiani G, et al.(2013) Intravesical glycosaminoglycans in the management of chronic cystitis. Minerva Urol Nefrol 65(4): 249-262.

5. Bayrak O, Seckiner I, Solakhan M, Karakok M, Erturhan SM, et al,(2012) Effects of intravesical dexpanthenol use on lipid peroxidation and bladder histology in a chemical cystitis animal model. Urology 79(5): 1023-1026.

6. Niku SD, Stein PC, Scherz HC, Parsons CL (1994) A new method for cytodestruction of bladder epithelium using protamine sulfate and urea. J Urol 152(3): 1025-1028.

7. Donmez M I (2016) The Early Histological Effects of Intravesical Instillation of Platelet-Rich Plasma in Cystitis Models. Int Neurourol J 20(3): 188-196.

8. Mayo ME, Hinman F (1976) Structure and function of the rabbit bladder altered by chronic obstruction or cystitis. Invest Urol 14(1): 6-9.

9. Soler R, Bruschini H, Freire MP, Alves MT, Srougi M, et al. (2008) Urine is necessary to provoke bladder inflammation in protamine sulfate induced urothelial injury. J Urol 180(4): 1527-1531.

10. Levin RM, Schuler C, Leggett RE (2016) Effect of Jack Bean Supplement on the Response of Rabbits to In-Vivo Experimental Cystitis (abstract). Taiwan Continence Society.

11. Carlini CR Ligabue-Braun R (2016) Ureases as multifunctional toxic proteins: A review. Toxicon 110: 90-109.

12. Staniscuaski F, Carlini CR (2012) Plant ureases and related peptides: understanding their entomotoxic properties. Toxins (Basel) 4(2): 55-67.

13. Olivera-Severo D, Wassermann GE, Carlini CR (2006) Ureases display biological effects independent of enzymatic activity: is there a connection to diseases caused by urease-producing bacteria? Braz J Med Biol Res 39(7): 851-861.

14. Follmer C, Real-Guerra R, Wasserman GE, Olivera-Severo D, Carlini CR (2004) Jackbean, soybean and Bacillus pasteurii ureases: biological effects unrelated to ureolytic activity. Eur J Biochem 271(7): 1357-1363.

15. Yao Y, Xuzhen Cheng, Lixia Wang, Suhua Wang, Guixing Ren (2011) Biological potential of sixteen legumes in China. Int J Mol Sci 12(10): 7048-7058.

16. Sowndhararajan K, Siddhuraju P, Manian S (2011) Antioxidant activity of the differentially processed seeds of Jack bean (Canavalia ensiformis L. DC). Food Science and Biotechnology 20: 585–591.

17. Akpapunam MA, Sefa-Dedeh S (1997) Jack bean (Canavalia ensiformis): nutrition related aspects and needed nutrition research. Plant Foods Hum Nutr 50(2): 93-99.

18. Spettel S, De E, Elias T, Schuler C, Leggett RE, et al. (2012) Citrate synthase, sarcoplasmic reticular calcium ATPase, and choline acetyltransferase activities of specific pelvic floor muscles of the rabbit. Mol Cell Biochem 370(1-2): 1-5.

19. Guven A, Lin WY, Leggett RE, Kogan BA, Levin RM, et al. (2007) Effect of aging on the response of biochemical markers in the rabbit subjected to short-term partial bladder obstruction. Mol Cell Biochem 306(1-2): 213-219.

20. Nigro DA, Haugaard N, Wein AJ, Levin RM (1999) Metabolic basis for contractile dysfunction following chronic partial bladder outlet obstruction in rabbits. Mol Cell Biochem 200(1-2): 1-6.

21. PhD MG, (2016)Understanding Cystitis: Everything you need to know about cystitis - from symptoms to solutions. E-Books: Natural Health Practice. 20.

22. Quigley AF, Kapsa RM, Esmore D, Hale G, Byrne E (2000) Mitochondrial respiratory chain activity in idiopathic dilated cardiomyopathy. J Card Fail 6(1): 47-55.

23. Levin RM (1999) Metabolic factors influencing lower urinary tract function. Exp Physiol 84(1): 171-194.

24. Hsu, TH, Levin RM, Wein AJ, Haugaard N (1994) Alterations of mitochondrial oxidative metabolism in rabbit urinary bladder after partial outlet obstruction. Mol Cell Biochem 141(1): 21-26.

25. Levin RM, Haugaard N, Wein AJ (1992) Metabolic alterations induced by obstructive hypertrophy of the rabbit urinary bladder. Jpn J Pharmacol 58 Suppl 2: 341P.

26. Levin RM, Hypolite JA, Haugaard N, Wein AJ (1996) Comparative response of rabbit bladder smooth muscle and mucosa to anoxia. Neurourol Urodyn 15(1): 79-84.

27. Hypolite JA, Longhurst PA, Gong C, Briscoe J, Wein AJ, et al.(1993) Metabolic studies on rabbit bladder smooth muscle and mucosa. Mol Cell Biochem 125(1): 35-42.

28. Callaghan CM, Johnson A, Neumann P, Leggett RE, Schuler C, et al. (2013) The effect of partial outlet obstruction on calpain and phospholipase-2 activities: analyzed by severity and duration. Mol Cell Biochem 381(1-2): 217-20.

29. Zhao Y, Levin SS, Wein AJ, Levin RM(1997) Correlation of ischemia/reperfusion or partial outlet obstruction-induced spectrin proteolysis by calpain with contractile dysfunction in rabbit bladder. Urology 49(2): 293-300.

30. Radu F, Leggett RE, Schuler C, Levin RM (2011)The effect of antioxidants on the response of the rabbit urinary bladder to in vitro ischemia/reperfusion. Mol Cell Biochem 355(1-2): 65-73.