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Hypericum perforatum remediates varicocele-induced reproductive failure in mice: Proof of concept in vitro evidence

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Abstract

Varicocele (VL) is closely linked with male infertility. This experimental study was executed to examine the effect of Hypericum perforatum (HP) on epididymal sperms traits and in vitro fertilizing potential, as well as early embryonic development in a murine model of VL. Twenty-four mature male mice were randomly allotted to four groups (n=6), including control, sham, VL, and VL with HP (100.00 mg/kg/day; PO). After 35-day treatment period, testicular total anti-oxidant capacity (TTAC), epididymal sperm characteristics, and in vitro pre-implantation embryo development were assessed. Experimental VL caused marked reductions in TTAC, and sperm quantity and quality, as well as poor in vitro fertilization (IVF) outcomes compared to the control and sham groups. Interestingly, HP administration uplifted TTAC, along with spermatological parameters and IVF success rate in comparison with VL group. These findings indicate that HP can redress VL-associated reproductive dysfunction in mice through building up anti-oxidant defense machinery.

Keywords: Anti-oxidant, Hypericum perforatum, In vitro fertilization, Mice, Varicocele

Introduction

Reportedly, varicocele (VL) as the most common remediable cause of male-factor infertility has been recorded in 35.00-44.00% of men with primary infertility and 45.00-81.00% of men with secondary infertility.[1] Anatomically, VL being characterized by abnormally dilated veins of the pampiniform plexus is generally left-sided; however, bilateral VLs are also quite common. Besides, it has long been revealed that unilateral VLs cause bilateral testicular malfunction.[2]

Although numerous prior studies have suggested various mechanisms by which VL induces testiculopathy and subsequent reproductive impairments, the obvious etiological factors underlying the development of VL-related male reproductive disorders remain largely unknown. Accordingly, it has been widely accepted that testicular thermodysregulation and blood-testis barrier disruption following VL-evoked oxidative stress (OS) and renal and adrenal metabolites reflux can lead to pathospermia, resulting in subfertility/infertility.[3]

A growing body of evidence has highlighted the unmistakable role of OS in pathophysiology of VL-elicited male-factor infertility. Correspondingly, it has been well-defined that OS causes sperm DNA disintegration, being the major clinical finding in patients experiencing VL-associated infertility.[4]

In line with that, anti-oxidant therapy has been propounded as a promising strategy to reinvigorate anti-oxidant defenses against OS complications. Hypericum perforatum (HP), a perennial plant being native to Asia and Europe, has been used as a medication for 2000 years. It has been reported to possess a variety of pharmacological properties, including anti-bacterial, anti-depressant, wound-healing, and anti-viral effects, owing its bioactive compounds, such as phloroglucinol derivates (hyperforin and adhyperforin), flavonoids (rutin, hyperoside, isoquercitrin, and quercitrin), biflavonoids (biapigenin and amentoflavone), and naphthodianthrones (hypericin and pseudohypericin).[5] It has been well-detailed that HP effectively impedes OS-mediated apoptosis, DNA damage, and inflammatory responses.[6] Further, empirical evidence indicates that HP has a potent protective effect in reproductive disorders via anti-oxidant defense machinery reinforcement along with anti-inflammatory activities.[7]

Hence, the objective of the current study was to uncover the effects of HP on testicular OS, as well as epididymal sperms traits and in vitro fertilizing potential, and early embryonic development in a murine model of VL.

Materials and Methods

Animals

Twenty-four mature male mice (Age: eight weeks; weigh: 28.20 ± 4.90 g), were used in this study. The animals were provided from the Animal Resources Center, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, and housed in an environmentally controlled room (temperature: 21.00-24.00 ˚C and 12 hr light/12 hr dark) with free access to food and water. All experiments in this study were conducted in accordance with the guidance of Ethical Committee for Research on Laboratory Animals of Urmia University, Urmia, Iran.

Experimental protocol

Following two-week acclimation, mice were allocated to four groups (n=6) as follows:

Control group: Mice received no medication, sham group: The abdominal cavity was opened; however, there was no VL induction, VL group: Abdominal cavity was opened; animals underwent VL induction and received no medication, and VL + HP group: Abdominal cavity was opened; animals received HP (Kneipp® Johanniskraut Dragees H; 100.00 mg/kg/day)[6] orally for 35 days following VL induction.

Surgery

All surgical procedures were implemented under anesthesia (60.00 mg/kg 10.00% ketamine hydrochloride and 10.00 mg/kg 2.00% xylazine hydrochloride; IP). Renal vein diameter was reduced to one mm; left renal vein ligation was made at a direct medial to adrenal and spermatic veins junction. After that, the anastomotic branch between the left testicular vein and left common iliac vein was ligated. The ligature was made around the probe, the probe was removed, and the vein allowed expanding within the boundary of the ligature, leading to a reduction in the renal vein diameter to one-half (Figure 1).[8]

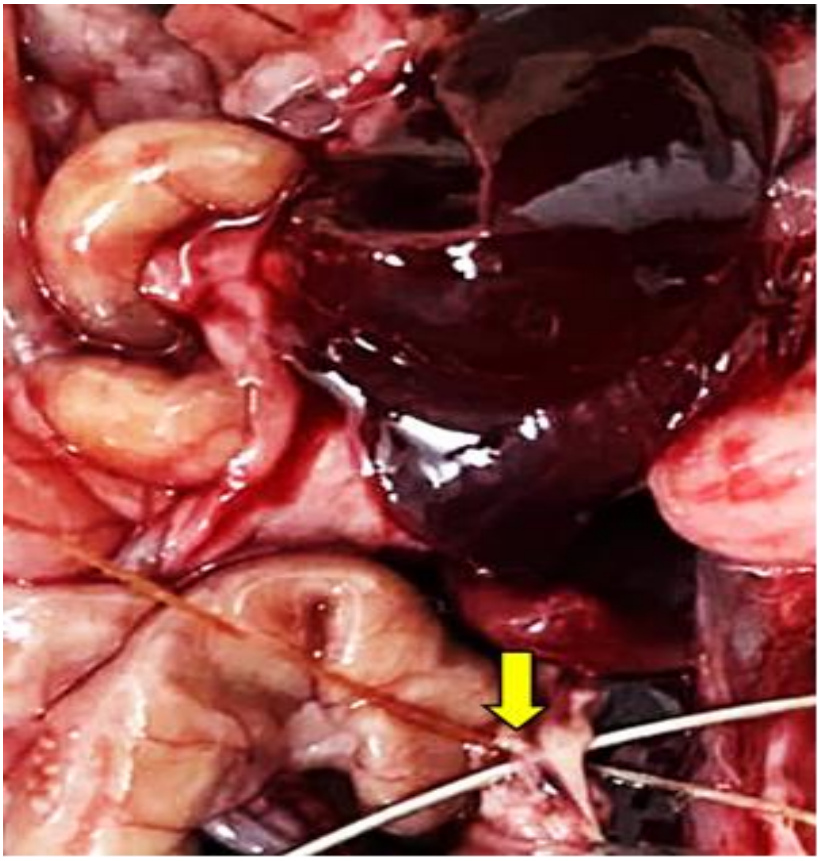

Figure 1. Experimental murine model of varicocele. The tunnel around the renal vein (arrow) was dissected and partial ligation of left renal vein was made.

Spermatology

Sperm concentration was determined using hemocytometer following epididymal sperms collection through slicing the caudal region of the epididymis into small pieces in 1.00 mL of human tubal fluid (HTF) + 4.00 mg/mL bovine serum albumin (BSA) and incubation for 30 min at 37˚C in 5.00% CO_2. results were expressed as millions of sperm/mL.[9]

Sperm viability was assessed using eosin/nigrosin staining. For each sample, 200 sperm cells were viewed at ×400 magnification, and the percentage of alive sperms (unstained) was recorded.[10]

The percentage of epididymal sperms with rapid progressive forward, slow progressive forward, and circumferential movements in 10 microscopic fields were also computed to determine sperm motility.[9]

In vitro fertilization (IVF)

The capacitated epididymal sperms (1.00×10^6 /1.00 mL HTF) were added to the HTF+BSA medium containing oocytes being dissected out from oviducts ampullae of super-ovulated female mice, and fertilization rate was recorded after four to six hours through observation of two pronuclei. Then, blastocysts formation was determined following four to five days culture of zygotes being transferred into the fresh pre-equilibrated medium.[11]

Testicular total anti-oxidant capacity (TTAC)

The TTAC was measured in homogenized testicular tissue using biochemical assay kit (Naxifer™; Navand Salamat Co., Urmia, Iran) according to the manufacturer's instructions.

Statistics

Statistical analyses were made using one-way ANOVA in SPSS Software version 22. All data were expressed as the mean ± standard deviation, and the $p<0.05$ was considered to be statistically significant.

Results

Spermatology

Spermatological analyses revealed that VL caused pronounced ($p<0.05$) reductions in epididymal sperms concentration, motility, and viability compared to the control and sham groups. While, HP treatment led to marked ($p<0.05$) improvements in spermatological parameters in comparison with VL group (Table 1).

Table 1. Spermatological parameters in different experimental groups.

Groups	Sperm count (10^6/mL)	Sperm motility (%)	Sperm viability (%)
Control	**60.04±3.47[a]**	**73.11±3.71[a]**	**83.47±5.87[a]**
Sham	**60.21±2.39[a]**	**69.82±4.09[a]**	**79.85±5.09[a]**
Varicocele	**33.57±4.09[b]**	**41.29±4.07[b]**	**55.86±6.07[b]**
Varicocele + Hypericum perforatum	**51.23±3.14[c]**	**59.91±3.96[c]**	**69.05±6.32[c]**

[abc] Different superscript letters indicate significant differences ($p<0.05$) between groups in the same column.

Fertilization rate and early embryonic development

As shown in Table 2, experimental VL resulted in significant ($p<0.05$) decreases in fertilization rate and blastocysts formation compared to the control and sham groups. Interestingly, epididymal sperms in vitro fertilizing potential and blatulation rate were significantly ($p<0.05$) increased in VL + HP group compared to the VL one.

Testicular total anti-oxidant capacity level

The TTAC significantly ($p<0.05$) reduced following VL induction in comparison with control and sham groups. Whereas, in VL + HP group, TTAC significantly ($p<0.05$) increased compared to the VL group (Table 2).

Table 2. Fertilization rate, embryonic development, and testicular total anti-oxidant capacity (TTAC) in different experimental groups.

Groups	Fertilization rate (%)	Blastocysts (%)	TTAC (mmol/L)
Control	**88.03±5.36[a]**	**63.94±7.03[a]**	**1.13±0.03[a]**
Sham	**81.23±6.48[a]**	**59.97±6.23[a]**	**1.11±0.01[b]**
Varicocele	**61.17±6.77[b]**	**39.91±5.86[b]**	**0.56±0.02[b]**

Varicocele + Hypericum perforatum	**77.89±5.91^{c}**	**49.86±7.27^{c}**	**0.99±0.02^{c}**

^{abc} Different superscript letters indicate significant differences ($p<0.05$) between groups in the same column.

Discussion

In the present study, it was found that mice epididymal sperms quantity and quality were influenced negatively by VL. Moreover, our findings revealed that VL-induced sperm damages in mice led to poor IVF outcomes and developmental retardation of pre-implantation embryos. Consistently, it has been reported previously that patients with VL have higher rates of sperm cells damage.[4]Additionally, it has been well-documented that damaged sperms are associated with pregnancy losses in humans and animals.[12]Accordingly, earlier reports have also stated that VL can cause sperm cells damage, consequently affecting males' fertility potential.[13]

Our results also exhibited that VL incapacitated mice testicular anti-oxidant defense system. Likewise, multiple former studies have shown that VL-mediated OS in testicular tissue can cause sperm count, motility, and vitality reduction, along with increases in sperm DNA fragmentation and morphological defects through testicular microenvironment alteration.[14]Furthermore, it is well-founded that VL-induced OS in testicular tissue can disrupt blood-testis-barrier and steroidogenesis through affecting Leydig and Sertoli cells, leading to impaired spermatogenesis.[15]

To address these challenges, anti-oxidants have been introduced to alleviate VL-connected reproductive disorders. In agreement with prior reports, our findings demonstrated that HP administration improved VL-related reproductive dysfunction in mice through testicular anti-oxidant defense system invigoration. Analogously, recent evidence suggests that astaxanthin, a xanthophyll carotenoid with potent anti-oxidant properties, can be beneficial as an adjuvant therapy for infertile men following varicocelectomy due to the anti-oxidant status modulation.[16] Lately, it has also been implied that carvacrol, a phenolic monoterpene having anti-oxidant activities, can be used as a therapeutic agent for VL-related infertility regarding its anti-oxidant and testiculo-protective effects.[17]Concordantly, ellagic acid, a bioactive and pharmacologically active polyphenolic compound, has shown to subdue VL-linked sperm cells damage and early embryonic development failure in mice due to its pronounced anti-oxidant potential.[18]

In accordance with the observed repro-protective effects of HP in the current study, it has been reported that HP exerts protective effects against testicular ischemia/reperfusion injuries in rats,[19] and cyclophosphamide-induced reproductive toxicity in mature BALB/c male mice.[20]

Conclusion

In Toto, in can be deduced that HP could remediate VL-associated reproductive dysfunction in mice through testicular anti-oxidant defense machinery fortification, owing its pharmacological activities,

particularly anti-oxidative effect. Further pathway-oriented experimental and clinical studies are needed to unveil all aspects of HP treatment in VL cases.

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