Trop J Nat Prod Res, January 2026; 10(1): 6704 - 6707

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effects of Electroacupuncture Combined with Standard Therapy on DNA Fragmentation Index and Testosterone in Men with Varicocele

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ARTICLE INFO

Article history:
Received 11 December 2025
Revised 10 January 2026
Accepted 16 January 2026
Published online 01 February 2026

ABSTRACT

Varicocele is a common cause of male infertility and is associated with oxidative stress, impaired sperm DNA integrity, and altered reproductive hormones. This study evaluated whether adding electroacupuncture (EA) to standard therapy is associated with improvements in sperm DNA fragmentation index (DFI) and serum testosterone in infertile men with varicocele. We conducted a single-blind randomized controlled trial at the Sekar Fertility Clinic, Dr. Moewardi General Hospital, Surakarta, Jawa Tengah, Indonesia, from April to October 2023. Fourteen participants were randomized into two groups: the control group received standard therapy (*O-(hydroxyethyl)-rutosides* and *Tribulus terrestris*) while the intervention group received the standard therapy combined with EA twice weekly for 6 weeks. Serum testosterone (ng/dL) and DFI (%) were assessed before and after treatment. Testosterone increased in both groups. However, the intervention group demonstrated a significantly greater increase (393.90 ± 140.11 ng/dL) compared to the control group (60.14 ± 63.52 ng/dL, $p < 0.05$). DFI decreased significantly within the intervention group (from 26.06 ± 9.36% to 15.40 ± 6.71%, p=0.011), while the change in the control group was not significant (p=0.145). Given the small sample size and the absence of EA-only, these findings should be considered preliminary and warrant confirmation in larger trials.

Keywords: Electroacupuncture, Deoxyribonucleic Acid fragmentation index, male infertility, testosterone level, varicocele.

Introduction

Male infertility is a global health issue and contributes to approximately 40-50% of infertility cases in couples.[1] This disorder is generally associated with abnormalities in sperm analysis parameters, such as oligozoospermia (reduced sperm count), asthenozoospermia (decreased motility), and teratozoospermia (abnormal morphology).[2] One of the most common causes of male infertility is varicocele, with a prevalence of about 15–20% in the general male population and found in nearly 40% of infertile men.[3]

Oxidative stress plays a critical role in the impairment of spermatogenesis, primarily due to increased levels of reactive oxygen species (ROS).[4] Increased ROS leads to sperm DNA integrity issues and is linked to increased DNA fragmentation, which is associated with germ cell apoptosis and deterioration in overall sperm quality.[4] Factors such as heat exposure, toxins, smoking, and alcohol consumption further exacerbate oxidative stress, highlighting the need to address these influences in the context of varicocele-related infertility.[5]

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Citation: Uki R Budihastuti, Bhisma Murti, Teguh Prakosa, Ida Nurwati, Abdurahman Laqif, Eriana Melinawati, Metanolia Sukmawati, Agung S Wijayanti, Ilham Ramadhanis, Faika Oesmania. Effects of Electroacupuncture Combined with Standard Therapy on DNA Fragmentation Index and Testosterone in Men with Varicocele. Trop J Nat Prod Res. 2026;10 (1):6704 – 6707 https://doi.org/10.26538/tjnpr/v10i1.38

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Electroacupuncture (EA), a combination of electrical stimulation and conventional acupuncture, is reported to have antioxidant, anti-inflammatory, neuroprotective, and anti-apoptosis effects.[6] This intervention can decrease ROS production and improve sperm DNA stability, potentially reducing DNA fragmentation and improving fertility parameters.[7] Furthermore, previous studies have also stated that acupuncture is able to increase levels of reproductive hormones, including testosterone, in infertile men.[8]

However, varicocele-related infertility is multifactorial, involving not only oxidative damage but also impaired microcirculation and hormonal disturbances.[9,10] Therefore, a combined therapeutic approach may be more rational than a single-modality intervention. *O-(hydroxyethyl)-rutosides*, as a vascular protectant, possess strong antioxidant properties. Studies have demonstrated that this compound effectively scavenges free radicals, thereby mitigating oxidative damage.[11] *Tribulus terrestris* has been widely investigated as a phytotherapeutic agent for male reproductive disorders and is thought to support spermatogenesis partly through modulation of the hypothalamic–pituitary–gonadal (HPG) axis, including effects on gonadotropins and Leydig-cell steroidogenesis, increased testosterone production and improved fertility-related parameters.[12,13] Several studies indicate that *Tribulus terrestris* plays a role in improving parameters such as sperm motility, viability, and overall count.[14,15] Moreover, research highlights its ability to positively influence reproductive hormone levels, including testosterone, which can be particularly beneficial for patients experiencing hormonal disturbances.[16–18] Previous studies indicate that treatments combining acupuncture with other adjunct therapies show promising results in improving fertility outcomes, emphasizing the potential for enhanced treatment efficacy through such a combination.[19]

Although acupuncture has been explored in male infertility and varicoceles, evidence is limited regarding the efficacy of adding EA to a "standard therapy" regimen. Studies directly comparing standard

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

therapy alone versus standard therapy plus EA, with outcomes focused on DFI and testosterone, are limited. Therefore, this study aimed to compare changes in DFI and serum testosterone between infertile men with varicoceles receiving standard therapy (*O-(hydroxyethyl)-rutosides* and *Tribulus terrestris*) plus EA and those receiving standard therapy alone.

Materials and Methods

Research Design

This study used a single-blind randomized controlled trial with a pre-test–post-test control group design. The research was conducted at the Sekar Fertility Clinic of Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia, from April to October 2023.

Ethical Approval

This research obtained ethical approval from the Health Research Ethics Committee of Dr. Moewardi General Hospital, number: 704/V/HREC/2023. All participants signed a written consent before participating in the study.

Research Subject

The participants of the study were an infertile men with a diagnosis of varicocele. Sample selection was random sampling from the population that met the inclusion criteria (men aged 20–35 years diagnosed with varicocele, defined by ultrasonography showing pampiniform plexus diameter >3 mm with venous reflux, participants also had at least one abnormal semen parameter (oligozoospermia, asthenozoospermia, and/or teratozoospermia), and exclusion criteria included azoospermia or severe oligozoospermia (<5 million/mL), alcohol consumption during the study period, contraindications to EA (e.g., pacemaker, local infection, allergy to materials), and inability to complete the intervention protocol.

A total of 14 participants who met the criteria were divided into two groups: the intervention group (n=7) and the control group (n=7).

Interventions

Standard therapy (i.e. both groups received the standard therapy):

O-(hydroxyethyl)-rutosides was administered orally in the form of a film-coated tablet containing 300 mg per tablet (PT Teguhsindo Lestaritama (Indonesia), No. TR132374571), taken twice daily (total daily dose 600 mg) for 6 weeks.

Tribulus terrestris was administered orally in the form of a film-coated tablet containing 275 mg per tablet (PT Teguhsindo Lestaritama (Indonesia), No. TR152588141), taken twice daily (total daily dose 550 mg) for 6 weeks.

Intervention group (i.e. received the Standard therapy and EA)

In addition to the standard therapy, participants received EA twice weekly for 6 weeks (12 sessions).

EA Procedure:

Acupuncture points: CV6 (Qihai), ST29 (Guilai), SP6 (Sanyinjiao), ST36 (Zusanli), KI3 (Taixi), and LI4 (Hegu).

Supine position of the patient – the action is carried out aseptically. Using a sterile disposable acupuncture needle of size 0.25×25 mm or 0.25×40 mm. The piercing was done gently until it reaches deqi, a sensation typical of acupuncture.

Electrical stimulation with Hwato SDZ-V (Shanghai, China), 2 Hz frequency, duration 15–30 minutes.

EA frequency 2 times/week for 6 weeks (12 sessions in total).

All procedures were conducted by competent health workers in the field of EA.

Also, in the control group (standard therapy only) the participants received standard therapy without EA.

Randomization and Blinding

Randomization was done using computer software to ensure a balanced distribution. Outcome testers (testosterone levels and DFI) were blinded to the group's allocation. Participants were not informed about the group they were following (single-blind).

Variable Measurement

DNA Fragmentation Index (DFI):

DFI was measured using the Sperm Chromatin Dispersion (SCD) test (SpermFunc DNAf). Semen samples were obtained by masturbation after 2–7 days of sexual abstinence. The semen concentration was standardized to 5–10 × 10^6 sperm/mL. A total of 60 µL of semen was mixed with melted agarose and maintained at 37°C. Microscope slides were pre-cooled at 2–8°C for 5 minutes, after which 30 µL of the prepared sperm suspension was placed onto the slide, covered with a coverslip, and incubated at 2–8°C for 5 minutes to allow solidification of the agarose microgel. After gel formation, the coverslip was gently removed, and the slide was treated sequentially with Solution A (acid denaturation solution containing hydrochloric acid) for 7 minutes, followed by Solution B (lysis solution containing dithiothreitol and Triton X-100) for 25 minutes at room temperature. The slide was then dehydrated in graded ethanol (70%, 90%, and 100%) for 2 minutes each, air-dried, and stained using Wright's stain (15–20 drops) to enhance contrast for microscopic evaluation. Slides were examined under a light microscope (CX33, Olympus Corporation, Japan) at 400× magnification (40× objective). At least 500 spermatozoa were evaluated per sample. DFI was expressed as the percentage of spermatozoa showing DNA fragmentation.

Serum Testosterone:

Serum testosterone was measured from venous blood samples collected from the antecubital vein using standard aseptic technique. Approximately 0.5 mL of blood was transferred into a disposable serum tube. After clotting, samples were centrifuged to obtain serum and analyzed using the enzyme-linked immunosorbent assay (ELISA) method. Testosterone concentrations were reported in ng/dL. Measurements were performed before and after the intervention.

Statistical Analysis

Data were analyzed using IBM SPSS statistical software version 25. The data was considered to be significant at the level of $p<0.05$.

Results and Discussion

A total of 14 participants were included (intervention n=7, control n=7). Baseline characteristics showed that there was no significant difference between the two groups ($p > 0.05$), indicating that the initial conditions of the participants were homogeneous. Most participants were aged 31–35 years, had a duration of infertility <10 years, normal body mass index (BMI), were non-smokers, and reported no erectile dysfunction (Table 1).

Within-group comparisons showed a significant reduction in DFI in the intervention group ($p=0.011$), whereas the reduction in the control group was not statistically significant ($p=0.145$) (Figure 1). Both groups demonstrated statistically significant increases in serum testosterone after treatment. However, the magnitude of the increase was larger in the intervention group than in the control group (Figures 2). This study showed that combination therapy, EA+standard therapy, resulted in a more significant increase in testosterone levels in infertile men with varicocele compared to the control group that received only standard therapy. The increase in testosterone levels in the intervention group reached 393.90 ± 140.11 ng/dL ($p<0.05$), while in the control group it was only 60.14 ± 63.52 ng/dL ($p<0.05$). This improvement suggests that EA+standard therapy may contribute to the regulation of reproductive hormones. This is in line with several previous studies that have shown that acupuncture is able to increase testosterone secretion through stimulation of the neuroendocrine system.[6,8]

Varicocele is known to interfere with Leydig cell function through increased scrotal temperature, testicular hypoxia, and the accumulation of ROS, leading to decreased testosterone production.[20,21] EA works by stimulating afferent nerve fibers that modulate the hypothalamic–pituitary–gonadal axis (HPG axis), thereby increasing LH secretion and testosterone stimulation.[8,22]

In addition, EA reduces oxidative stress and increases testicular perfusion through vasodilation mechanisms as well as activation of the antioxidant pathway ERK/Nrf2/HO-1, which allows Leydig cells to maintain optimal testosterone production.[19,23] A systematic review and meta-analysis showed that non-pharmacological interventions, including transcutaneous electrical acupoint stimulation at a frequency of ~2 Hz and EA, ranked highest in increasing testosterone hormone in

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

oligoasthenospermia patients.[24] Similarly, clinical research suggests that electroacupuncture can improve the symptoms of premature ejaculation and that such changes may be related to the regulation of testosterone levels.[25]

Table 1: Characteristics of research subjects

Characteristics	Group		p-value
	Intervention (n=7)	Control (n=7)	
Age (years)			
20-25	0 (0.0%)	0 (0.0%)	**0.051**
26-30	0 (0.0%)	3 (42.9%)	
31-35	7 (100.0%)	4 (57.1%)	
Duration of infertility (years)			
<10	4 (57.1%)	5 (71.4%)	**0.577**
>10	3 (42.9%)	2 (28.6%)	
BMI			
Normal	5 (71.4%)	4 (57.1%)	**0.577**
Obesity	2 (28.6%)	3 (42.9%)	
Smoke			
No	6 (85.7%)	4 (57.1%)	**0.237**
Yes	1 (14.3%)	3 (42.9%)	
Education			
High School	2 (28.6%)	2 (28.6%)	**1.000**
College	5 (71.4%)	5 (71.4%)	
Work			
Civil servants	1 (14.3%)	1 (14.3%)	**0.753**
Self-employed	3 (42.9%)	2 (28.6%)	
Private sector employee	3 (42.9%)	3 (42.9%)	
Unemployed	0 (0.0%)	1 (14.3%)	
Erectile dysfunction			
No	5 (71.4%)	6 (85.7%)	**0.515**
Yes	2 (28.6%)	1 (14.3%)	

BMI; body mass index

The results of this study also showed a significant decrease in DFI in the intervention group compared to the control group (p=0.018). A decrease in DFI from 26% to 15.4% indicated improved spermatozoan DNA stability after intervention. This is important because a decrease in DFI correlates with improving sperm quality, as sperm DNA fragmentation can reduce sperm motility and fertility and negatively impact reproductive success.[26,27] In varicoceles, an increase in ROS leads to damage to the sperm DNA chain through lipid peroxidation and disruption of chromatin remodeling in the seminiferous tubules.[28]

EA plays a role in lowering testicular oxidative stress by improving local blood flow, increasing oxygen supply, and decreasing ROS production.[29] In addition, previous research has also shown that EA can stimulate the hypothalamic-pituitary-gonad, which is a key pathway in the regulation of male reproductive hormones.[30] EA plays a role in lowering ROS by inhibiting sperm lipid peroxidation and improving testicular blood flow, both of which can help improve sperm parameters in men with varicocele.[28]

A study by Ketabchi & Salajegheh [31] reported that the combination of EA with varicoclectomy resulted in significant improvements in motility, morphology, and DFI compared to varicoclectomy alone. Other studies and meta-analyses also confirm that acupuncture is one of the most effective modalities for improving sperm quality parameters in male infertility.[30,32,33]

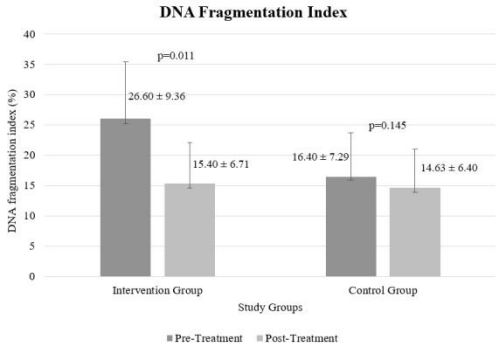

Figure 1: Comparison of DFI Before and After Intervention

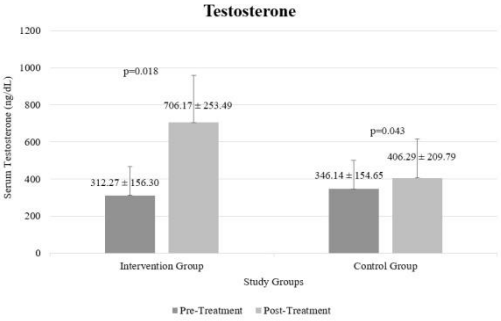

Figure 2: Testosterone Comparison Before and After Intervention

Although the results of this study are promising, there are several limitations to consider, including the relatively small sample size that limits the power of statistical tests.

The sample size (n=14) limits statistical power. These limitations may affect the generalization of the results, so further research with larger sample sizes and longer intervention durations is needed to reinforce these findings. Secondly, the absence of an EA-only arm prevents attribution of effects specifically to EA. Despite these limitations, the findings suggest that adding EA to standard therapy may be associated with improved DFI and a larger rise in testosterone compared with standard therapy alone. Larger, adequately powered trials incorporating additional control arms (EA-only and/or sham EA) and standardized hormone sampling protocols are needed to confirm efficacy and clarify the mechanisms underlying hormonal and DNA integrity changes.

Conclusion

In infertile men with varicocele, standard therapy plus EA was associated with a significant reduction in DFI and a greater increase in testosterone compared with standard therapy alone. However, these findings should be interpreted as preliminary and exploratory, given the small sample size and the absence of an EA-only group. Future research should consider larger multicenter trials with standardized hormone assay procedures and appropriate control arms to confirm the method effectiveness and understand the mechanisms of the hormonal and DNA integrity variations.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
The authors would like to thank Universitas Sebelas Maret for institutional support. This work was supported by Universitas Sebelas Maret on Mandatory Research (MR-UNS) funding number 228/UN27.22/PT.01.03/2023.

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