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Impact of Quercetin on Oxidative Stress and Sperm Function in Leukocytospermic Subfertile Men

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ABSTRACT

Background: Leukocytospermia, which is defined as the substinence of a raised amount of white blood cells in seminal fluid, is generally correlated with male subfertility. It causes oxidative stress, influencing the motility and morphology of sperm and their DNA integrity. The objective of the study is to investigate the association between leukocytospermia (as a marker of oxidative stress) and impaired seminal constraints in subfertile men, and to evaluate the role of quercetin natural antioxidant, in taming sperm quality, motility, and overall outcomes of fertility.

Methods: This case-control study was conducted for a duration of 6 months and included a total of 30 male participants, comprising 15 subfertile men with Leukocytospermia and 15 healthy fertile controls. Participants were recruited using a purposive sampling technique from the infertility clinic of Imran Idrees Teaching Hospital and the general community in Sialkot. Semen samples were collected following a standardized abstinence period of 3–4 days Data were analyzed using SPSS version 21, and group comparisons were made using independent t-tests, with a significance level set at $p < 0.05$.

Results: Subfertile men had significantly lower sperm count (30.71 ± 1.65 vs. 75.67 ± 5.46 million/mL; $p<0.001$), decreased motility (52.47 ± 5.61% vs. 78.33 ± 2.70%; $p=0.017$), and increased leukocyte counts (3.75 ± 0.45 vs. 0.51 ± 0.04 million/mL; $p<0.001$) than controls. Levels of Quercetin were significantly decreased in subfertile men and negatively correlated with leukocytospermia ($p<0.001$).

Conclusion: Leukocytospermia is associated with oxidative stress and compromised sperm quality. Quercetin is promising in inhibiting oxidative damage and enhancing fertility in subfertile men. More research is advised to confirm its clinical efficacy.

Keywords: Antioxidants, Leukocyte count, Male Infertility, Oxidative Stress, Quercetin, Reactive Oxygen Species (ROS).

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INTRODUCTION

The worldwide fall in fertility levels is emerging as a rapidly evolving public health issue. Infertility now affects about 08–15% of couples globally, and in as much as 30% of these cases, male factors are the main culprits. Male infertility is clinically described by the absenteeism of conception following a year of unprotected coitus[1]. This condition is an expression of numerous disorders of the male reproductive system and is often manifested by deviant semen constraints, including abridged sperm concentration, meager motility of sperm, and anomalous morphology[2]. The increasing incidence of male infertility emphasizes the need to investigate its multifactorial etiology and possible therapeutic approaches.

The etiology of male infertility is multifactorial and often complex. It encompasses a broad range of factors, including lifestyle practices (smoking, alcohol consumption, physical inactivity), dietary deficiencies, chronic systemic conditions such as diabetes or varicocele, endocrine disturbances, genetic defects, environmental and occupational exposure to heat, radiation, and toxins[3]. Further, infectious and inflammatory diseases of the male reproductive system significantly contribute to worsening semen quality. Of these, leukocytospermia, a condition categorized by an excessive count of white blood cells (leukocytes) in the semen, has been of interest due to its direct correlation with subfertility[4].

According to the World Health Organization (WHO), leukocytospermia is a condition where over 1×10^6 leukocytes per milliliter of semen are present. It occurs in 30% of subfertile men and is much common in this group than in fertile men, in whom it is seen in about 10–20% of the population. Leukocyte infiltration into seminal fluid is most often caused by infections, inflammation, or urogenital tract disease[5]. Though leukocytes are valuable immune system components, their surplus amount in semen is harmful because of the disproportionate production of reactive oxygen species (ROS). These compounds, if not sufficiently neutralized by antioxidant defense mechanisms, create a condition called oxidative stress (OS) [4,5].

Oxidative stress is a substantial factor involved in male subfertility. ROS are very volatile particles that can destroy cellular components such as lipids, proteins, and nucleic acids. ROS overproduction overwhelms the seminal plasma antioxidant ability and causes a direct dysfunction in sperm motility and morphology. Spermatozoa are specifically prone to oxidative mutilation because of the small number of cytoplasmic contents and high concentration of polyunsaturated fatty acids in the plasma membrane. Lipid peroxidation, motility defect in sperm, morphological defects, DNA fragmentation, and consequently resulting in lower fertilization capacity[6].

In men with leukocytospermia, this pathological redox environment is also amplified by the pro-inflammatory cytokines secreted by activated leukocytes. These pro-inflammatory mediators also inhibit antioxidant mechanisms and continue to cause tissue destruction, creating a vicious cycle that degrades the sperm[7]. Therefore, control of oxidative stress has emerged as a key therapeutic goal in male infertility, especially complicated by leukocytospermia.

Among the broad spectrum of antioxidants that have been investigated, quercetin has received significant interest due to its strong biological activities. Quercetin is a naturally occurring polyphenolic flavonoid found in onions, apples, berries, and leafy vegetables[8]. It possesses several activities, such as antioxidant, anti-inflammatory, anti-apoptotic, and anti-carcinogenic activities[9]. In male fertility, quercetin has been shown to scavenge ROS, suppress lipid peroxidation, and stabilize mitochondrial membranes. These actions cumulatively contribute to sperm function and integrity preservation. Clinical and experimental evidence indicates that supplementation with quercetin can enhance semen quality, increase sperm motility, and decrease DNA damage in men with oxidative stress-induced infertility[8,9].

Even with the increased evidence for quercetin's antioxidant activity, its particular contribution in leukocytospermia-induced oxidative stress cases remains poorly considered. Though other research has attempted to examine its benefits within oxidative stress state as a whole, few works are aimed towards addressing the inflammation and immunologic aspects of leukocytospermia and whether quercetin can cross-talk with them[10].

Beyond this, nothing much is understood about the dose-response relationship, bioavailability, or mechanisms of how quercetin acts in such sperm cells among this target group.

Elucidating the molecular and cellular processes underlying leukocytospermia is crucial in the development of focused therapeutic interventions. Since ROS-mediated sperm damage is a reversible cause of infertility, direct antioxidant approaches to neutralize oxidative stress are promising. Quercetin, through modulation of oxidative and inflammatory mechanisms, is a possible adjunct treatment in semen improvement and improvement of fertility outcome[10].

This research was intended to explore the interaction between leukocytospermia and oxidative stress in subfertile men, particularly the potential protective effect of quercetin. Through comparison of seminal parameters and quercetin content between fertile controls and subfertile men with leukocytospermia, we intend to define the connection between leukocyte-mediated oxidative damage and male fertility. Understanding this connection may provide a scientific foundation for antioxidant-based therapies and help establish quercetin as a viable candidate for managing oxidative stress-related subfertility. The objective of the study is to investigate the association between leukocytospermia (as a marker of oxidative stress) and impaired seminal constraints in subfertile men, and to evaluate the role of quercetin, a natural antioxidant, in taming sperm quality, motility, and overall outcomes of fertility.

METHODS

This case-control study was done after clearance from the Institutional Review Board (IRB) of Imran Idrees Teaching Hospital, Sialkot, Pakistan (IRB#: 2023/IITH/RA/0018) for six months from 13th Nov 2023 till April 2024. All the subjects gave written informed consent after they were fully apprised about the aims, procedure, and confidentiality policy of the study.

Purposive Sampling Technique was used. A total of 30 male participants were enrolled and categorized into two groups: Group A (Controls, n = 15) included healthy, fertile males, and Group B (Subfertile, n = 15) included men diagnosed with leukocytospermia and subfertility. Using OpenEpi software, the sample size was calculated at a 95% confidence level and with sufficient statistical power to detect significant differences in seminal quercetin levels between groups[11]. Control group inclusion criteria were healthy married men with documented fertility, either by natural conception or by documented continued pregnancy of their wives. For the subfertile group, inclusion was restricted to men aged 25–40 years, married for over one year, with no pregnancy despite regular unprotected coitus, and presenting to the infertility clinic for assessment. All subjects in this group had documented leukocytospermia on semen analysis.

Exclusion criteria were men with azoospermia, history of systemic disease, hormonal disorders, known chromosomal or genetic abnormalities, testicular surgery or trauma, and obstruction of the urogenital tract. Also excluded was any case in which the female partner was diagnosed with an absolute cause of infertility to reduce confounding variables. Other exclusions were individuals on antioxidant or hormone therapy in the last three months to prevent interference with research findings.

Participants provided semen samples after abstaining from sexual activity for 3–4 days. Samples were left to liquefy at 37°C and analysis of semen was executed according to World Health Organization (WHO) 2020 standards, including evaluation of sperm concentration, morphology, and motility. Kruger's strict morphological criteria were employed for the assessment of sperm morphology.

Leukocytospermia was then confirmed with the Endtz test, wherein samples with a round cell count $>0.2 \times 10^6$/mL were further tested to ascertain the leukocytic origin. A cut-off of $>1 \times 10^6$ white blood cells/mL semen was used to define leukocytospermia.

Quercetin concentrations in semen were quantified by High-Performance Liquid Chromatography (HPLC) on a Hitachi Primaide Organizer System (Tokyo, Japan) with a UV detector at 370 nm. Calibration was performed with analytical-grade quercetin standards from Sigma-Aldrich, and method validation was conducted with semen samples spiked with known concentrations of quercetin. Analytical accuracy and reproducibility were ascertained, with an intra-assay coefficient of variation <5%.

All the data were apprehended and assessed with SPSS version 21.0 (IBM Corp., Armonk, NY). Data are expressed as Mean ± Standard Error of Mean (SEM). Statistical comparisons between groups were resolved using the Independent Samples T-test, and a p-value <0.05 was used to express statistical significance.

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RESULTS

Table 1: Anthropometric Characteristics of Study Participants

Parameter	Fertile Males (Mean ± SEM)	Subfertile Males (Mean ± SEM)
Age (years)	29 ± 1.456	32 ± 1.548
Height (cm)	156.24 ± 7.291	166.33 ± 1.904
Weight (kg)	65.03 ± 3.89	72.80 ± 2.16
BMI (kg/m ^{2})	28.90 ± 2.58	26.24 ± 0.38

Table 1 shows the anthropometric characteristics of the study participants. Subfertile males had a higher mean age (32 ± 1.55 years) compared to fertile males (29 ± 1.46 years). Their average height was 166.33 ± 1.90 cm, while fertile males measured 156.24 ± 7.29 cm. Mean weight was 72.80 ± 2.16 kg in subfertile males and 65.03 ± 3.89 kg in fertile males. The BMI was 26.24 ± 0.38 kg/m² in subfertile males and 28.90 ± 2.58 kg/m² in fertile males.

Figure 1 indicates that the sperm count among subfertile males was less (30.71 ± 1.65 million/mL) than controls (75.67 ± 5.46 million/mL; $p < 0.001$). Sperm motility in subfertile males was less (52.47 ± 5.61%) than controls (78.33 ± 2.70%; $p = 0.017$).

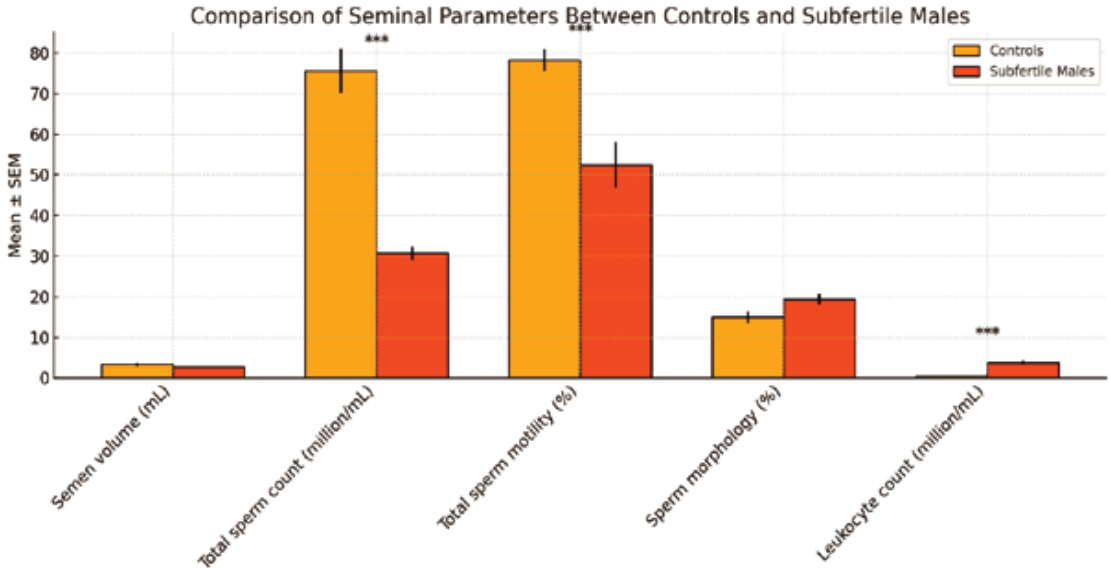

Figure 1: Comparison of Seminal Parameters between healthy controls and Subfertile men

Figure 2 indicates the Relationship of Leukocytospermia and Quercetin in controls and subfertile males. It was observed that the Leukocyte count was increased in subfertile males (3.75 ± 0.45 million/mL) compared to controls (0.51 ± 0.04 million/mL; $p < 0.001$). Quercetin levels were decreased in subfertile males, with a mean difference being -739.42 ng/mL ($p < 0.001$). There was an inverse correlation between leukocyte count and quercetin levels.

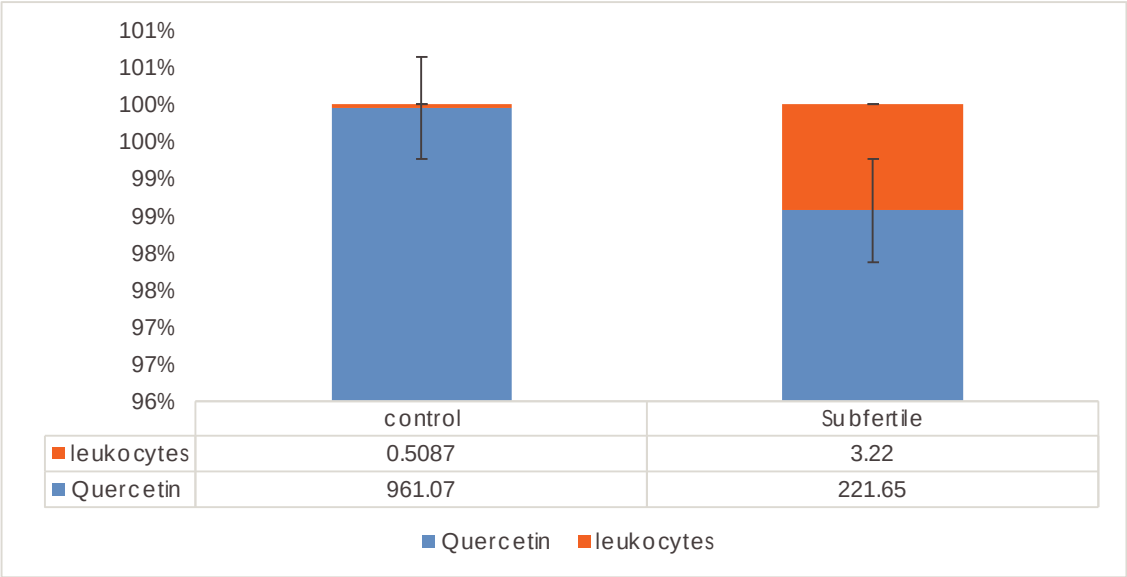

Figure 2: Relationship of Leukocytospermia and Quercetin Among Controls and Subfertile Males

Table 2: Inverse Relationship of Quercetin with Leukocytospermia Controls and Subfertile Males

Variable	Assumption	Levene's Test F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval (Lower - Upper)
Leukocytospermia	Equal variances assumed	15.615	<0.001	8.625	28	<0.001	-2.71133	0.31435	-3.35526 to -2.06741
Leukocytospermia	Equal variances not assumed.	-		8.625	14.394	<0.001	-2.71133	0.31435	-3.38383 to -2.03884
Quercetin	Equal variances assumed	15.584	<0.001	4.674	28	<0.001	739.41600	158.19687	415.36440 to 1063.46760
Quercetin	Equal variances not assumed.	-		4.674	15.103	<0.001	739.41600	158.19687	402.42839 to 1076.40361

Table 2 shows an independent samples t-test, which disclosed substantial differences in both leukocytospermia and quercetin levels between the groups. For leukocytospermia, the test showed a statistically significant reduction ($t = -8.625$, $p < 0.001$) with a mean difference of -2.71, and a 95% confidence interval extending from -3.38 to -2.04. For quercetin, a significant increase was observed ($t = 4.674$, $p < 0.001$), with a mean difference of 739.42 and a confidence interval of 402.43 to 1076.40. In both cases, Levene's test specified unequal variances ($p < 0.001$), and results were interpreted accordingly.

DISCUSSION

The present study evaluated the contribution of quercetin in subfertile men with leukocytospermia and found significant variations in seminal parameters, such as sperm count, motility, and leukocyte count. These results are consistent with existing literature suggesting that leukocytospermia is a contributing factor for increased oxidative stress, which destroys sperm function through reactive oxygen species (ROS) production and fragmentation of DNA [12].

Our results indicated significantly lower seminal quercetin concentrations in subfertile men than in fertile controls, with a significant inverse correlation between leukocyte number and quercetin content. This finding is consistent with earlier studies highlighting quercetin's antioxidant function in neutralizing

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ROS and promoting sperm motility and viability[12,13]. Comparable antioxidant effects were seen in sperm cryopreservation models, where quercetin supplementation enhanced motility, inhibited lipid peroxidation, and maintained DNA integrity under oxidative stress conditions[14].

Whereas sperm motility and count were severely affected among the subfertile group, sperm morphology remained unaffected. This, to some extent, differs from other studies in which abnormal morphology is reported to be associated with leukocytospermia [15,16]. This variation may occur because of limited sample size, the intensity of inflammation, or due to variance in morphological assessment criteria.

Additionally, existing literature indicates that oxidative stress may remain independent of leukocytospermia. Indeed, static oxidation-reduction potential (sORP) and DNA fragmentation of sperm have also been found to be elevated in subfertile men deprived of leukocytospermia [17,18,19]. This indicates that although leukocytospermia is a primary source of ROS, other intrinsic conditions like immature sperm, varicocele, or environmental exposure could also contribute to the oxidative semen environment[20].

Quercetin's capacity to quench ROS, stabilize mitochondrial activity, and defend sperm DNA from fragmentation is widely documented throughout species and models [21,22,23]. Nonetheless, a few studies highlights that its antioxidant effect depends on the dosage and can become pro-oxidative at certain doses or through extended exposure [24,25,26]. Thus, thorough consideration of its therapeutic window becomes critical.

While our research does validate a protective effect of quercetin for sperm quality, limitations are that the sample size is relatively small, there is no longitudinal follow-up, and other antioxidants such as glutathione or vitamin C are not included for comparison. Future research should investigate synergistic effects of combinations of antioxidants and clinical pregnancy outcomes to more accurately establish quercetin's reproductive potential.

The current research sheds light on the interplay between oxidative stress and male infertility, with special reference to leukocytospermia. By revealing a substantial negative relationship between leukocyte number and seminal quercetin content, the results emphasize the significance of antioxidant depletion among subfertile men and the potential value of quercetin as both a biomarker and a drug candidate. These findings reinforce the expanding body of evidence that antioxidant imbalance is a significant cause of poor sperm function and is potentially an amendable variable in the treatment of male subfertility. Clinical relevance emerges in the potential for the use of quercetin, an inexpensive and ubiquitous antioxidant, within treatment protocols directed towards the augmentation of sperm quality and, ultimately, possibly improving fertility.

Several limitations to this study should be noted. The fairly modest sample size constrains the generalizability of the results and diminishes the power to identify subtle but clinically significant differences. The cross-sectional design does not allow any inferences regarding causality between quercetin depletion and reduced fertility outcomes. Furthermore, the investigation was conducted only on quercetin, without measuring other antioxidants like glutathione, vitamin C, or catalase, which would have given a better overview of the oxidative status. The absence of sperm DNA fragmentation data also constrains the mechanistic insight into ROS-induced damage. Moreover, the lack of follow-up information on clinical endpoints like pregnancy or live birth rates also limits the translational relevance of the findings to actual fertility success.

In the future, larger and more heterogeneous studies will be required to confirm these observations and determine the reproducibility of the findings across populations. Longitudinal studies would help establish whether boosting quercetin levels with supplements translates into favorable fertility outcomes down the line. Future studies ought to also be accompanied by an expanded panel of oxidative and antioxidative biomarkers to further capture the seminal redox environment. Randomized, controlled trials addressing quercetin's clinical efficiency, ideal dosing, as well as its safety profile are needed before any recommendation in customary clinical practice can be made. In addition, research on the combined action of quercetin with other antioxidants can uncover synergistic effects. Investigating these areas will not only establish the position of quercetin in the treatment of oxidative stress-related subfertility but also open the door to individualized antioxidant therapy in male infertility treatment.

CONCLUSION

The findings of this study support the potential role of quercetin as a protective antioxidant capable of improving sperm quality in men with elevated leukocyte counts. While promising, these results warrant further investigation through larger, controlled studies to establish clinical relevance and therapeutic efficacy.

LIST OF ABBREVIATIONS

ROS: Reactive Oxygen Species
OS: Oxidative Stress

WHO: World Health Organization
HPLC: High-Performance Liquid Chromatography
SEM: Standard Error of Mean

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CONFLICT OF INTEREST
The authors declare no conflict of interest related to this study.

ETHICAL APPROVAL
This study was approved by the Institutional Review Board (IRB) of Imran Idrees Teaching Hospital, Sialkot, Pakistan (IRB#: 2023/IITH/RA/0018). All participants provided written informed consent according to the Declaration of Helsinki.

AUTHORS' CONTRIBUTION
QUA was the primary author with the idea and objective for the study. **SR and FS** assisted in designing the methodology and reviewing the questionnaire. **NH** helped in ensuring data confidentiality and assisted with data analysis, while **HM and AI** contributed to data entry and supported the manuscript write-up and analysis.

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