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Beneficial impacts of natural antioxidants on molecular structure and parameters of human sperm

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Abstract

Background: Sexual dysfunctions are often associated with oxidative stress, DNA damage, and reduced levels of sex hormones.

Methods: In this study, a combination of polyfloral honey, royal jelly (RJ), Korean ginseng, and palm pollen were tested to reduce oxidative DNA damage in semen. The study involved 25 male patients aged 22-45 years who were participating in a fertility clinic in Wasit, South-East Iraq .Surgically resolvable problems were excluded, as well as subjects who smoked.

Results: Semen analysis and serum total oxidant status were used to assess the fertility potential in males, and pregnancy occurrence served as evidence of sperm ability for fertilization. Our findings indicate that consuming honey with Korean ginseng and palm pollen improved sperm count by reducing caspase-3 activity and protecting against oxidative DNA damage. In addition, forward progressive motility and morphology of the sperm were significantly improved by the treatment and also had a direct effect on mitochondrial enzymes.

Conclusion: Therefore, the use of honey combined with specific herbals appears to be a promising treatment option for the improvement of sperm parameters to treat male infertility.

Introduction

There can be a variety of reasons why a man fails to conceive, including abnormalities in his semen, anatomical, endocrine, genetic, functional, and immunological abnormalities of the reproductive system, and chronic illnesses of the reproductive system. Although medical treatments have been introduced for this condition, their efficacy remains uncertain [1]. Male infertility is identified as the cause of approximately more than half of infertile couples who cannot conceive, Throughout the world, infertility is prevalent among Iranian couples attempting to conceive for two years being found to be 8% [2-4]. There is a growing incidence of male reproductive problems. Reactive Oxygen Species (ROS) play a role in intracellular pathways that modulate the activation of different transcription factors in sperms [3]. Higher levels of ROS have been shown to stimulate sperm function, but antioxidant molecules may interfere with physiological sperm function [5]. The sperm head comprises three main parts: the acrosome, the post-acrosomal lamina, and the nucleus, with the acrosome containing hydrolytic enzymes necessary for the sperm to penetrate an oocyte [6].

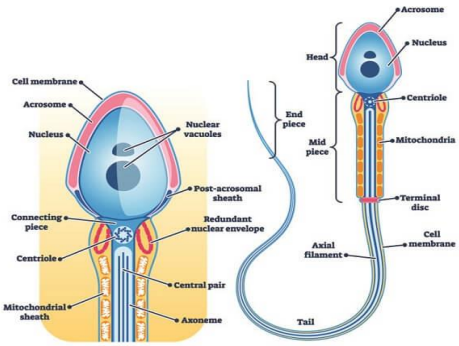

Figure 1: Anatomy of spermatozoa.

Honey is produced by bees using a combination of nectar and secretions from plants and insects [7,8]. It is a mixture of plant and animal materials known for its sweet taste, nutritional value, and medicinal benefits [8,9]. Honey contains sugars, water, organic acids, and polyphenols that act as antioxidants. The phenolic compounds in honey vary depending on its geographical location and source [10]. The chemical composition and quality of honey depend on several factors, including botanical source, seasonality, location, honeybee metabolism, and processing techniques [6,10,11]. Royal jelly is a substance produced by worker bees with various health benefits, including antioxidant, antibacterial, and anti-inflammatory effects [11,12]. Ginseng is a traditional herbal medicine derived from the Panax plant root and has several bioactive substances that improve overall health, increase energy, and enhance the immune system. Ginseng may also aid spermatogenesis, improve testicular damage, and enhance sperm quality and mobility [13-17]. Male infertility is traditionally treated with date palm pollen, claimed to have aphrodisiac and fertility-enhancing properties [18]. It contains amino acids, vitamins, minerals, and fatty acids that contribute to its nutritional value. A study investigated the efficacy of palm pollen suspensions and other herbal remedies with various concentrations on male infertility [18-22].

As part of our study, we aim to examine the effects of natural antioxidants on the post-acrosomal lamina, head enzymes, and sperm parameters in infertile males, Similarly, RJs, KRGs, and DPPs were administered orally to determine the effect on semen analysis of honey and herbal cocktails, and the molecular structure of sperm heads and hydrolytic enzymes. The study of the mechanism of honey, RJ, KRG, and DPP against oxidative stress and seminal fluid has not been clearly studied. Therefore, our study focuses on the potential impact of this mix in preventing reproductive complications in men.

Methods

The Huma reader (ELISA) method was used to perform serum total oxidant status (TOS) measurements, following the kit assay protocol developed by Bioassay Technology Laboratories, China. 0.02 mL of semen was well mixed and added with 0.38 mL of the prepared test solution. From the above mixture, 1-2 mL of the solution is placed on Neubauer chamber and counted large 2 squares. The formula calculates the total oxidant number by multiplying the number of sperm by 100,000 (in millions per milliliter).

The study enrolled 25 male patients aged between 22-45 years who visited the infertility Clinic in Wasit, a southeast region of Iraq. A fertility specialist evaluated all participants for a comprehensive diagnosis. Interviews and questions were conducted with the patients concerning their sexual behavior, medical histories, and family histories of infertility, Moreover, the subjects were asked about prior surgical interventions, childhood diseases such as cryptorchidism that may affect fertility, and prior drug use such as wine, beer and tobacco. Additionally, their exposure history to chemicals and ionizing radiation was acquired. Infertility issues that could be solved surgically were excluded from all participants due to a full urological examination [23-26]. There were abnormal sperm counts, motility parameters, or morphologic parameters in the study's infertile men who were non-alcoholic, non-drug abusers, without genitourinary abnormalities, and did not use drugs that

affect spermatogenesis. The serum total oxidant status (TOS) was measured using the Huma reader (ELISA) method and Bioassay Technology laboratories, **in**China provided the kit assay [27-28].

The study included three main steps. The herbal cocktails were prepared for the treatment [24]. A total of 40 mixes were made for 40 individuals, with each mix containing 1 Kg of poly floral honey, 15 gm of royal jelly (RJ), 20 gm of Korean red Ginseng Powder (KRG), and 100 gm of Dates palm pollen Powder (DPP) [29-33]. The main treatment involved the administration of polyfloral honey and herbal cocktails (RJ, KRG, and DPP) twice a day for two months, with each dose containing 25 gm [34-36]. Sperm collection and examination were conducted to the evaluation of the sperm parameters after the treatment.

Masturbation was used to obtain semen samples in the hospital laboratory from the participants who had abstained from sexual activity for 48-72 hours. Semen collection was performed twice, both prior to and following the two-month therapy period [38-40]. The Sperm Quality Analyzer was used to conduct seminal analyses, which included volume, number of sperm, sperm motion, sperm structure, and sperm progressing activity [37]. A high-resolution light microscope was used to analyze sperms in order to determine the final motile sperm quantities and shape. The motility of each spermatozoon was graded based on its progressive motion. The data was obtained utilizing the SAS application to perform statistical analysis, and the significant differences between means were determined using the T-test (Analysis of Variation-ANOVA). Based on the outcomes of the sperm analysis, the treated group was prepared for insemination.

Results

The trial involved 40 infertile men in total, all of whom finished the prescribed course of treatment and conducted a subsequent semen examination. The patients ranged in age from 22 to 42 years, with a mean age of 31.20 ±5.15. The effect of the mixture on epididymal sperm parameters was evaluated and presented in Table 1. The results demonstrated a significantly significant ($p<0.001$) rise in sperm morphology following the administration of the mixture for two months as opposed to before treatment. There was no noticeable difference in sperm volume after treatment. However, the number of sperm rose up to 6.5*106 mLG1. Every other day for two months, infertile patients treated with the mixture demonstrated a substantial improvement in sperm motility (p 0.05) and sperm activity (4.3%). There was also a significant improvement in sperm rapid motility. The mixture had some effect on the PUS cell, RBC, and PH, as shown in Table 1.

Parameters	Before treatment	After treatment	P-value
Semen volume (mL)	3.92 ±1.87	3.27 ±1.40	0.247
Total count *10^6 mL^{-1}.	35.10 ±19.51	39.05 ±19.69	0.682
Sperm active	11.11 ±6.76	15.94 ±12 .01	0.146
Sluggish	29.73 ±13.99	26.55 ±10.95	0.448
Normal-form	61.05 ±21.25	63.94 ±18.97	0.661
Abnormal form	29.47 ±11.65	30.78 ±13 .25	0.747
Rapid Mot	3.57 ±2.56	8.00 ±7.09	0.0497 *
Liner Mot	10.17 ±8.17	9.23 ±6.86	0.737
PUS	2.12 ±1.16	2.27 ±1.40	0.717
R.B.C	1.93 ±1.09	1.31 ±1.10	0.251
PH	7.32 ±1.87	7.46 ±1.46	0.818
* ($P \leq 0.001$). NS: Non-significant.			

SD: standard deviation, values are given in means ± SD.

Table 1: Before and after treatment with honey and herbal extract, infertile men's mean values, standard deviations, and percentages of various variables were compared.

Sperm analysis

The results indicate the overall oxidative state, measured in U/ml, before and after treatment across different age ranges. Among individuals aged 22 to 30 years, the mean overall oxidative state before treatment was 14.75 U/ml, with a standard deviation of 1.99. Following treatment, there was a notable reduction in the overall oxidative state to 4.33 U/ml, with a standard deviation of 1.00. Similarly, for the age group of 31 to 38 years, the mean oxidative state before treatment was 15.11 U/ml (±1.27), decreasing to 4.65 U/ml (±0.95) after treatment. Individuals aged 39 to 45 years exhibited a mean overall oxidative state of 13.38 U/ml (±1.65) before treatment, which decreased to 4.36 U/ml (±0.97) after treatment. These findings suggest a consistent trend of reduced oxidative state following the administered treatment across all age groups, highlighting its potential efficacy in mitigating oxidative stress.

Discussion

The study suggests that the addition of honey to herbal cocktails improves sperm parameters, particularly, the measure of the total motility of sperm and the grade of its forward movement. The sugars in honey, such as fructose and glucose, the minerals potassium, magnesium, calcium, sodium chloride, sulfur, ferrous, zinc, phosphates, and the vitamins C, B1, B2, B3, B5 and B6, are all essential to stimulating sperm motility. Honey also contains enzymatic and non-enzymatic antioxidants by reducing reactive oxygen species (ROS) damage caused by oxidative stress. After treatment, normal morphological sperm showed no significant differences between the groups [41-43]. The addition of royal jelly to honey also improved sperm parameters due to its antimicrobial and antioxidant properties [44]. protein, carbs, lipids, vitamins, free amino acids, and mineral salts are just a few examples of the bioactive compounds that are found in. 83% to 90% of the proteins in royal jelly (RJ) are major royal jelly proteins (MRJPs) and these components supported the results of increased sperm activity, normal form, and reduced sluggishness [45-48]. The study also found that ginseng

promotes spermatogenesis and increases the motility and morphology of epididymal sperm [46-48]. However, it was not effective in changing the concentration of sperm. Ginseng may work through its antioxidant properties, but more research is needed to determine its exact mechanism [49,50]. Finally, estradiol and flavonoid substances found in date palms have beneficial impacts on sperm quality [51-53]. DPP, a component of date palm, has scavenging properties that improve sperm parameters [54]. The study found that concentrations of DPP up to 100 mg/kg showed the best effects on sperm parameters. Overall, the study suggests that these herbal cocktails can improve male reproductive activity and competence.

It's interesting to note that the consumption of DPP suspension has been shown to increase seminal vesicle size in men, potentially due to the presence of gonadotropin-like substances or steroidal components in the DPP [55-57]. However, it was also observed that the volume of semen decreased in at least 25% of the test group who were treated with the herbal mixture. This could be due to the presence of antibiotic-like components in the mixture, or other factors such as old age or exposure to a course of treatment. It's also worth noting that certain antibiotics, antidepressants like lithium, and marijuana use have been associated with a reduction in male ejaculate volume.

Based on the data presented in Sperm analysis:it can be noted that the honey and herbal extract, particularly *Panax Ginseng*, may be effective in improving male fertility by enhancing the quality of sperm. The study found that the treatment resulted in a significant decrease in TOS levels, which suggests a reduction in oxidative stress that can be harmful to sperm [58-60]. The imbalance between oxidants and antioxidants in semen has been identified as a possible cause of infertility, and the study suggests that the treatment may help restore balance and promote spermatogenesis. Furthermore, the study indicates that the compounds found in the honey and herbal extract may improve the function of sperm by positively affecting the enzymes in mitochondria [61]. The positive effects of the Date palm pollen suspension on sperm quality suggest that it may be a promising natural remedy for male infertility.

Various herbal extracts such as Ginseng, honey, and date palm pollen suspension appear to improve male fertility by reducing oxidative stress and improving sperm quality. In order to fully understand these mechanisms and determine the optimal dosages and durations of treatment, more research is needed.

The glutathione peroxidase (GPX) catalyzes hydrogen peroxide and organic peroxide reduction in semen, including that of phospholipid peroxides. In its active place, it contains selenium in the form of selenocysteine In sperm, it is stored primarily in mitochondria, but a nuclear form has also been discovered, which protects sperm DNA from OS damage and participates in the process of chromatin condensation [62] Also to protect human cells and organ systems against free radicals, a complex antioxidant system exists. Dietary and endogenous enzymatic and non-enzymatic antioxidants counteract the damaging effects of free radicals by various mechanisms, such as electron donation, catalytic removal, binding radicals and/or gene expression regulation. Together, antioxidants constitute an integrated defence against ROS. Only when the production of free radicals overwhelms the antioxidant defence and the oxidant/antioxidant imbalance shifts in favor of oxidants, does oxidative stress develop [63]. The importance and the use of the amino acid cysteine in the fight against ROS impacts the cell because it is a limiting substrate for glutathione synthesis. Cysteine (2 mM) and taurine (2 mM) (a cysteine-derived) antioxidant properties were controversial when they were used during the cryopreservation procedure of bull spermatozoa [64]. Different findings suggest that sperm mitochondria play a pivotal role in the decrease of sperm quality caused by ROS. In germ cells, mitochondrial proteins and membrane lipids are damaged, and mtDNA is fragmented. Therefore, ATP synthesis is severely affected, and the decrease in energy production results in meiotic arrest, causing the presence of abnormalities in sperm morphology. High ROS levels also disrupt mitochondrial membranes and induce apoptosis, thus leading to a decrease in sperm number [65].

Author Contributions

All authors share in the preparation and writing of the manuscript.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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