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Summary and Conclusion

Compromised fertility or infertility is a significant health issue in males and females. Various factors influence infertility in males. Genetic abnormality, endocrine disorders, testicular dysfunction, infections, environmental and occupational factors, and lifestyle practices are the leading causes of the poor quality of sperm. To understand the factors that lead to poor quality of sperm, the present study examined the influence of environment and lifestyle habits on sperm parameters.

For many years, traditional Indian medicine has utilized medicinal plants for various disease treatment purposes. *Rosa indica* is a species of rose that is native to the Indian subcontinent and having high antioxidant potential. Although some scientific evidence supports the potential health benefits of *R. indica*, its effect on male infertility is still not studied. Therefore, this study aimed to investigate the protective effect of *R. indica* petal extracts on male infertility.

The present research work was conducted in five phases. The Phase I was the retrospective study assessed the impact of environmental factors and lifestyle habits on semen parameters. Semen samples from 299 participants consulting for infertility to a private hospital in Coimbatore were collected and analyzed in accordance with WHO guidelines. The data included were age, sexual abstinence, the date of sample collection, addictions (alcohol consumption, smoking), and nature of the job (sitting hours). The season, addiction and the nature of job were correlated with sperm parameters using the SPSS package. There was a significant correlation between sperm parameters and lifestyle habits: season, abstinence and sitting hours. It was observed that individuals who sat for extended periods showed a significant decrease in semen volume (p-value 0.004), sperm count (p-value 0.01), and normal forms (p-value 0.039), increase in non-progressive (p-value <0.001) and nonmotile (p-value 0.037) sperms in comparison

to those who sat for less than 4 hours. While there are notable variations in sperm parameters across seasons, whereas, the observed values align with the standards set by the World Health Organization (WHO). However, the study did not establish statistically significant correlations between male infertility and addictions such as smoking and alcohol consumption.

In Phase II, the aqueous and ethanolic extracts of *R. indica* petals were analyzed for their *In vitro* anti-oxidant, anti-inflammatory and Cytoprotective properties. Both aqueous and ethanolic extracts fresh and dry petals showed the presence of alkaloids, flavonoids, phenols, sterols, anthocyanins and tannins. All the extracts exhibited a dose dependent DPPH, ABTS, hydroxyl, Nitric oxide, and hydrogen peroxide radical scavenging activity. The ethanolic extract from dry petals exhibited the highest radical scavenging with low IC50 value compared to aqueous petal extract.

Inflammation is a significant factor in male reproductive disorders. Oxidative stress caused by increased ROS and pro-inflammatory cytokines during heat stress harm spermatogenesis. The ethanolic extract of *R. indica* dry and fresh petal extracts exhibited dose dependent and maximum percentage of anti-inflammatory activity (59.96 ± 0.49 % and 57.27± 0.16 % respectively). Further they were efficient in maintaining the high percentage of cell viability of TM3 cell line even at the highest concentration of 200 µg/ml. The Phase II findings suggested that *R. indica* petals are a rich source for antioxidants that could be an effective medicinal agent in preventing the progress of oxidative stress.

In Phase III, both the aqueous and ethanolic extracts (fresh and dry petal extracts of 1:1 ratio were combined) were studied for their alleviating effects on steroidogenesis and spermatogenesis and their outcome on sperm quality in heat stress-induced male Wistar rats. After obtaining animal ethics clearance, the study was conducted on three months old male Wistar rats. Thirty animals were used for the present study in 5 groups, with six animals in each group for 28 days. Heat stress was induced by immersing hind legs, tail and scrotum in a 43°C thermostatically controlled water bath for 30 min for six consecutive days from day 8 of the treatment. The experimental groups included a control group (Group I), a

negative control group having heat-stress induced rats (Group II), a positive control group having heat-stress induced rats treated with 50 mg/kg quercetin (Group III), heat-stress induced rat with 200 mg/kg aqueous extract treated group (Group IV) and Heat stress induced rat with 200 mg/kg of ethanolic extract treated group (Group V). The Body weight, testis weight, Testosterone level, antioxidant properties of testis and semen parameters were analyzed at the end of the experiments.

A significant reduction in weight of the testis, testosterone level, antioxidant level, sperm concentration, motility and normal sperms in Group II experimental animals confirmed the effect of heat stress on sperm quality. Further, the histopathology of the seminiferous tubules showed germ cell aplasia, mild hyperplasia of Leydig cells and maturation arrest. Animals in Group III showed Leydig cells with 90 % normal spermatogenesis and Group V with 80%. The lipid peroxidation level was high in Group II animals. This indicate that the high level of lipid peroxidation destroys the lipid matrix in the sperm cell membranes which leads to loss of motility and impairment in sperm production. A significant increase in weight of the testis, testosterone level, antioxidant level, sperm concentration, motility and normal sperms in aqueous and ethanolic extract treated animals demonstrated the alleviating effect of the *R. indica* petal extract on heat stress induced impairment of spermatogenesis. Among the extracts the ethanolic extract showed sperm concentration, motility and normal forms of sperm almost on par with positive control.

Phase IV included the quantification of phytocompounds of *R. indica* petals extracts, followed by characterization of the extract using GC-MS and HPTLC analysis. The Phase IV results showed the presence of kaempferol, pyrogallol, and alpha tocopherol, plays a significant role in combating ROS in spermatozoa. The presence of lenolenate, a poly unsaturated fatty acid in the extract having major role in steroidogenesis. It helps to reduce ROS and increase serum testosterone level. Further, the presence of folic acid and astaxanthin, a major antioxidant used in improving male fertility was also present in the dry petal ethanolic extract.

The protective role of ethanol and aqueous extract of *R. indica* petals on lifestyle-induced oxidative stress on semen parameters was further validated using *in silico* approach in Phase V. All the 194 phytocompounds present in the extract were docked against the selected target proteins, namely, COX-2 (PDB ID: 5IKT), AR (PDB ID: 5VO4), AKT1 (PDB ID: 6HHF) and StAR proteins (PDB ID: 3P0L) using molecular docking software Glide, a module of Schrödinger release 2022-4. A total of 32 compounds were found to interact with all the target proteins with high negative GScore. Among these compounds, Kaempferol was identified as the top hit, exhibiting a GScore value of -9.455 for COX2, and -9.226 for AR. Whereas the GScore was -4.864 for StAR proteins. In the male reproductive system, the role of AKT1 in regulating spermatogenesis and promoting the survival of developing sperm cells while inhibiting apoptosis is crucial. Additionally, it involved in regulating StAR, which is essential for transporting cholesterol into the mitochondria of Leydig cells in the testis. Out of the compounds that were docked, only two compounds with PubChem ID 135369658 and 57387363 exhibited a significantly negative GScore against AKT1. Moreover, these compounds did not exhibit any interaction with allosteric amino acids. These findings suggest that these compounds could be potent activators of AKT1, which could be utilized to regulate sperm production. The results of this phase, reiterated the results observed in phase III studies.

Conclusion

The study found that prolonged sitting can increase temperature and disrupt thermoregulation, resulting in decreased testicular antioxidant, increased lipid peroxidation, seminiferous tubule deorganisation, damaged Sertoli and Leydig cells, spermatogenic arrest and ultimately, compromised semen parameters leading to male infertility. However, the phytocompounds present in the ethanolic extract of *R. indica* petals have a sequestering effect, which can alleviate heat stress induced male infertility by acting as potential antioxidants. While the findings suggest that *R. indica* could be useful in managing male reproductive ailments, further research and human trials are necessary to establish its potential as a therapy for male infertility caused by heat stress.

Recommendations for future studies

- The research can further be proceeded with proteomics analysis to find out the exact mechanism of heat stress on male infertility.

- Further validation of the identified compounds, such as Kaempferol and the compounds with PubChem ID 135369658 and 57387363, may be necessary to determine their potency in regulating sperm production.