

Abstract

Male infertility is a growing concern in India, with an estimated 12-18% of couples facing infertility issues. The present study was carried out with the aim of analysing the impact of lifestyle and environment on semen parameters and the protective effect of *Rosa indica* petal extracts on heat stress induced male infertility. The study was structured into five phases. In the first phase of the study, retrospective data from 299 male participants seeking medical assistance for fertility issues at a private hospital was analysed. The results of this phase showed a significant association between prolonged sitting (more than 4 hours per day) and semen parameters. 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.

The antioxidant, anti-inflammatory and cytoprotective potential of the fresh and dry petal extracts of *R. indica* was assessed in phase II. The ethanolic extract from dry petals exhibited the highest radical scavenging and anti-inflammatory activity with low IC₅₀ value compared to aqueous petal extract. Additionally, the cytoprotective test conducted with the MTT assay demonstrated the viability of TM3 cell lines even at the maximum concentration of 200 μ g/ml. Therefore, a dosage of 200 mg/kg was selected for *in vivo* studies in phase III. In this study, the male Wistar rats were exposed to heat stress by immersing hind legs, tail and scrotum in 43°C thermostatically controlled water bath for 30 min for 6 consecutive days from day 8 treated with a dosage of 200 mg/kg of equal ratio of fresh and dry petals of aqueous and ethanolic extracts, a positive control with quercetin (50 mg/kg), a negative control without treatment and a normal control to evaluate the alleviating effect of the extract on heat stress induced sperm abnormalities. The results showed a significant difference in the weight of the testis between the negative control (0.25 \pm 0.12 g) and different treatment groups (0.45 \pm 0.12 g in ethanolic extract and 0.43 \pm 0.11g in aqueous treated groups). The decline in body weight observed in the negative control group of animals from 301.5 \pm 2.15 g to 280.83 \pm 14.97 g could be attributed to factors such as heat stress. There was a substantial contrast in semen parameters between animals subjected with heat stress in negative control and those treated with extract, quercetin and control groups. The ethanolic extract-treated group showed an increased level of serum testosterone and the antioxidant levels (CAT,, GPx, GSH and SOD) compared to negative control group (p -value 0.05). The histopathology of the testis showed that the negative control group had germ cell aplasia, mild hyperplasia of Leydig cells, and maturation arrest. In contrast, the positive control group and the ethanolic extract treated groups showed normal spermatogenesis (90% and 80% respectively)..

The Phase IV studies focused on the identification of phytochemicals in both the extracts by GC-MS and HPTLC analysis. 194 compounds identified in phase IV were subjected to molecular docking studies using GLIDE (Schrodinger's module) in phase V to understand their interactions with key target proteins such as COX2, AKT1, StAR, and AR. Among 194 compounds, the Kaempferol a flavonoid and the phenolic compound 2,4-DTBP present in all the extracts showed interaction with all the target proteins studied. Kaempferol, alongside the presence of Vitamin E specifically in the ethanolic extract, could be a contributing factor in the restoration of normal spermatogenesis in the mice induced with heat stress and treated with ethanolic extract. Therefore, the present study clearly demonstrates the antioxidant and anti-inflammatory properties and the presence of kaempferol, Vit E and the phenolic compounds of the ethanolic extract of *R. indica* petals and its efficacy in alleviating heat stress-induced semen abnormalities in male Wistar rats.