

## Introduction

Infertility is a worldwide health problem affecting countless people of childbearing age. According to the latest research data from the World Health Organization (WHO, 2018), infertility affects approximately 48 million couples worldwide, of which the male factors contribute 40%. 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. Sperm count and quality are the significant factors for 90 % of male fertility problems. A systematic literature review shows the influence of various environmental and lifestyle factors such as smoking, alcohol consumption, drug use, obesity, sedentary life, stress, and sitting for longer than six hours a day reduce sperm parameters (Huang *et al.*, 2023).

Some lifestyle factors can disrupt hormonal imbalance, such as decreasing testosterone levels and affecting sperm production. Also, it affects the quality and quantity of sperm, such as sperm concentration, motility and morphology (Leisegang and Dutta, 2021). Though several factors contribute to male infertility, the present study focused on the most significant factors, such as genital heat stress.

The average sperm production is based on the optimal scrotal temperature maintained below body temperature. Low temperature affects the metabolic rate, the sperm is stored longer, and high temperature affects spermatogenesis (Kleisner *et al.*, 2010). Over the past thirty years, lifestyle habits such as occupation have been extensively studied as significant contributors to increased scrotal temperature and decreased male fertility. While the human body has effective mechanisms for maintaining normal cell homeostasis in response to internal and external factors, a disparity between endogenous antioxidants and reactive oxygen species (ROS) can lead to oxidative stress (OS).

ROS plays a significant role in OS by causing the oxidation of lipids, DNA, and protein by disrupting biological functions. Due to their unpaired electrons in

the outermost orbit, ROS are highly reactive molecules. Hydroxyl radical (OH•), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and the superoxide anion (O<sub>2</sub>•-) are the most common ROS, and they can be generated by leukocytes and any aerobic living cell, including spermatozoa (Cocuzza *et al.*, 2007). At physiological levels, ROS are necessary for different vital functions in the male reproductive system, including the assembly and regulation of tight junctions within the blood-to-testis barrier, signal transduction, production of male sex hormones, and other critical physiological changes of spermatozoa (Ashok *et al.*, 2014).

The higher concentrations of Poly Unsaturated Fatty Acids (PUFAs) in the sperm cell membrane and cytoplasm of sperm cells, as well as their low antioxidant capacity and poor DNA repair system, make them particularly vulnerable to ROS. However, ROS is also required for the maturation of sperm, capacitation, hyperactivation, acrosome reaction, and sperm-oocyte fusion. When ROS production exceeds the neutralizing capacity of antioxidants in seminal plasma, the detrimental effect of ROS can overwhelm the system (Battista *et al.*, 2008). The origin of ROS can be endogenous or exogenous, and the body's antioxidant mechanism aims to compensate the deleterious effects of these ROS molecules (Aranda-Rivera *et al.*, 2022; Gharagozloo *et al.*, 2011).

The body's natural antioxidant system plays a crucial role in maintaining a delicate redox balance by neutralizing high amount of ROS under normal aetiology. This system is composed of both enzymatic and non-enzymatic molecules, mainly found in the seminal fluid and spermatozoa. GPx, CAT, and SOD are the most important among the vital antioxidants (Qamar *et al.* 2023).

McLeod's paper, published in 1943, showed that increased oxygen tension leads to a decrease in sperm motility, which is reversed by catalase. The role of ROS in mediating sperm OS *in vivo* and *in vitro* was later confirmed when human sperm were exposed to a mixture of ROS caused by the xanthine oxidase system. A high level of ROS production is linked to impaired sperm function, and oxidative stress stimulates lipid peroxidation in sperm (Aitken, 2017).

Infertility in men can have significant psychological and social effects since they may be perceived as incomplete without the ability to father children. There are currently limited treatment options for male infertility, with available choices

including the use of medication to improve semen quality and assisted reproductive technique. Non-conventional medicine is commonly used by 70% to 80% of the population, as reported by the World Health Organization. As a result, herbal products have received increasing attention as a potential alternative treatment option for male infertility (Mishra *et al.*, 2018).

For many years, traditional Indian medicine has utilized medicinal plants for treatment purposes. According to the WHO, healing plants are the primary sources of healthcare for more than 75% of the world's population. These plants contain secondary metabolites, including glycosides, steroids, alkaloids, phenols, flavonoids, terpenoids, and glycoproteins, which have various potential benefits, including enhancing the quality and functionality of sperm cells (Roozbeh *et al.*, 2021).

*In vitro* studies play an important role in the evaluation of any activity and further support in *in vivo* studies. Animal models are very important in studying the effects of lifestyle on male fertility and the identification of active constituents in the plant material for the standardization of herbs and their formulations. *In silico*, which involves computer simulations and modeling, is becoming increasingly important in evaluating phytoconstituents after the characterization of plant materials. Further help to identify the active phytochemicals by predicting their molecular interaction and targeting pathways, predict pharmacokinetic and pharmacodynamic properties and enhance the understanding of the mechanism of action. There are drug candidates that were identified using *in silico* methods and have advanced to clinical trials (Papp *et al.*, 2012; Souers *et al.*, 2013).

With increasing knowledge on the role of antioxidants on OS and male infertility, there is an increase in the usage of antioxidants in treating male infertility (Silver, 2005). Due to the increased cost of synthetic antioxidants, antioxidants from plants are now of greater research interest (Silva *et al.*, 2022).

Several plants have been used to treat various diseases, including disease related to fertility, such as *Eurycoma longifolia* (Tambi *et al.*, 2012); *Cardiospermum halicacabum* (Peiris *et al.*, 2015); Grape seed extract (Bayatli *et al.*, 2013); *Syzygium aromaticum* (Gurib-Fakim 2006); *Nigella sativa* (Haseena *et al.*, 2015); *Lycium barbarum* (Luo *et al.*, 2006); *Tribulus terrestris* (Shalaby and

Hammouda 2014); *Asteracantha longifolia* and *Polycarpea corymbosa* (Mohan *et al.*, 2013); and *Taraxacum officinale* (Chung *et al.*, 2018).

*Rosa indica* belongs to the family Rosaceae (locally called as Rose). It is native to the Indian subcontinent and is commonly used in traditional medicine. It is a perennial herb. It is known for its sweet smell and beauty, generally offered to God and as decorative in the household. Besides that, the petals are rich sources of phytochemicals and are used in various herbal preparations (Pathak *et al.*, 2019; Zahid *et al.*, 2017; Rasheed *et al.*, 2015; Farook *et al.*, 2011; Hunt *et al.*, 1962)

In traditional Indian medicine, various formulations containing rose extracts are employed for their mild laxative, astringent, tonic, and antibacterial properties. They are used to address issues such as enlarged tonsils, sore throat, and gallstones, and are also valued for their cooling effects (Singh *et al.*, 1997). Rose essential oil derived from *R. damascena* is believed to possess analgesic and spasm-relieving properties (Sadraei *et al.*, 2013). Furthermore, rose extracts and their isolated compounds have been reported to exhibit antimicrobial, anti-HIV, and hypnotic properties (Basim and Basim, 2003; Karthy *et al.*, 2009). Additionally, it has been noted that when consumed as a food additive, rose oil can have a positive impact on various digestive tract disorders (Boskabady *et al.*, 2011). The rose petals have high antioxidant properties that help in curing many ailments (Pathak *et al.*, 2019; Zahid *et al.*, 2017; Cai *et al.*, 2005; Kumar *et al.*, 2006).

Limited research has been conducted on the phytoconstituents of *R. indica*. Bai *et al.* reported that the methanolic extract of *R. indica* petals contain quinic acid, 5-hydroxymethylfurfural, pyrogallol, levoglucosan, and 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (Bai *et al.*, 2015). The GC-MS revealed the presence of various chemical constituents, namely, acetic acid, mercaptohexyl ester, butanoic acid, 2-methyl-5-oxo-1-cyclopentene-1-yl ester, isosteviol, caryophyllene oxide, pentyl phenyl acetate, dihydromyrcene, 1,5-octadecadien, octadecanoic acid, ethyl ester, palmitic acid (2-phenyl-1,3-dioxolan-4-yl methyl ester), santolina epoxide, and 9-farnesene (Rasheed *et al.*, 2015). Similarly, another study indicated that the volatile oils in fresh *R. damascena* flowers

primarily consist of citronellol, geraniol, nonadecane, and heneicosane (Verma *et al.*, 2011).

A report by Tandon *et al.* (2018) on the effect of polyherbal formulation that included rose flowers on male infertility in rats found that the formulation improved sperm motility and viability, increased testosterone levels and reduced oxidative stress markers in rats. Although there is some scientific evidence supporting the potential health benefits of *R. indica*, its alleviating effect on male infertility is still not studied. Therefore, the present study was conducted to investigate the influence of lifestyle on male fertility and the protective effect of *Rosa indica* petal extracts on heat stress induced male infertility.

### Hypothesis

- The petals of *Rosa indica* are hypothesised to exhibit a substantial alleviating effect on male infertility induced by heat stress.

### Objectives:

The hypothesis was tested by framing the below mentioned objectives

- Assessing the impact of environmental factors and lifestyle habits on semen parameters
- Evaluating the *in vitro* antioxidant, anti-inflammatory and cytoprotective properties of aqueous and ethanolic extracts obtained from both dry and fresh petals of *R. indica*
- Evaluating the *in vivo* therapeutic effect of aqueous and ethanolic extracts of fresh and dry petals of *R. indica* on male Wistar rats subjected to heat stress
- Identifying the phytochemical constituents of *R. indica* petal extracts and validating their presence
- Conducting an *in-silico* analysis of the interactions between various phytochemical compounds found in the petal extracts of *R. indica* and specific targets associated with male infertility.

The literature relevant to the present study was reviewed in the second chapter.