



## EVALUATION OF *BRASSICA OLERACEA* EXTRACT ON STRESS INDUCED INFERTILITY IN ALBINO RATS

<sup>1</sup>Muskan Sharma, <sup>2</sup>Dr. Sunil Kumar Singh, <sup>3</sup>Vishal Kumar Yadav,  
<sup>4</sup>Meena bandiya, <sup>5</sup>Ritika Singh, <sup>6</sup>Anuj Dubey, <sup>7</sup>Yashwant Singh

<sup>1,3</sup>United Institute of Pharmacy, Naini, Prayagraj

<sup>2</sup>Professor, United Institute of Pharmacy, Naini, Prayagraj

<sup>4</sup>Assistant professor Ujjain institute of pharmacy Vikram University Ujjain

<sup>5</sup>Research Scholar, SMAS, Galgotias University, Greater Noida

<sup>6</sup>Research Scholar, Madhu Vachaspati institute of pharmacy, kaushambi

<sup>7</sup>Research scholar, Lords university Alwar Rajasthan

---

**Received:** 26/04/2023; **Accepted:** 08/05/2023; **Revised:** 17/05/2023; **Published:** 26/05/2023

---

### ABSTRACT

The principal objective of the study is to assess the manner in which *Brassica oleracea* var. *italica* (Broccoli) affects stress-induced infertility in male albino rats. Stress was administered using the Forced Swim Test (FST), and they received doses of 250 mg/kg and 500 mg/kg, respectively, of *Brassica oleracea* var. *italica* extract for 15 days and Sildenafil 5 mg/kg by mouth for 15 days, as standard medication. FST was performed one hour before the treatment. Animals were sacrificed on the sixteenth day using the cervical dislocation technique to assess infertility. SOD, CAT, and MDA were examined in biochemical parameters as oxidative stress markers. The lipid profile (total cholesterol, triglyceride, HDL, VLDL, and LDL) was also examined to assess lipid peroxidation as a result of stress. Both the initial and final body weights, as well as the weight of the reproductive organs, were measured on days 0 and 16. Semen characteristics including sperm count, motility, and viability are assessed, and a testosterone hormonal testing was also carried out. Our research is supported by the histological investigation of the testes, which also looked at spermatogonia, primary spermatocytes, and spermatids. *Brassica oleracea* var. *italica* also known as Broccoli.

Plants were subjected to a preliminary phytochemical assessment, and the detection of flavonoids, phenols, and tannins supports the antioxidant property. The extract of BOVI considerably improves both sperm quality and testosterone levels, and this effect is dose dependent for the treatment of stress-related infertility. In my conclusion, our research appears encouraging for the creation of herbal treatments for stress-related infertility.

**Keywords:** - *Brassica oleracea* var. *italica*, Forced Swim Test, stress induced infertility, lipid peroxidation, oxidative stress, sperm count, sperm motility and sperm viability, testosterone, spermatogonia, primary spermatocytes and spermatids.

---

DOI: 10.48047/ecb/2023.12.Si8.814

## 1.1 INTRODUCTION

**Definition:-** The World Health Organization defines infertility as the failure to get conceived even after no less than twelve months of consistent, sexual activity without protection. **Zegers-Hochschild et al. [38]** Being childless is considered as an alternative for infertility in underdeveloped and developing nations. **Cattapan et al. [10]** and infertility often referred to as carrying a healthy pregnancy and giving birth to a healthy child. **S.P. Aiswarya [3]** Around 186 million people experience infertility on a global scale. Infertility will continue to be predominantly a female social concern even if male infertility makes up more than 50% of all cases worldwide. **Inhorn et al. [19]**

Primary and secondary infertility are the two categories into which it is divided. A primary infertility couple is one who has never given the birth. Being unable to conceive after getting pregnant is known as secondary infertility. Stress is a real side effect of infertility. The severity of infertility is increasing with time due to technological advancements, increase use of devices that releases dangerous blue rays, disturbance in circadian rhythm etc.

## 1.2 Causes of Infertility

Men and women are infertile for a multitude of reasons. For instance, 5% of infertility can be attributed to inherited conditions, structural variations, and immunological or endocrinological issues. Many cases of infertility may also be significantly influenced by environmental

pollutants (such as heavy metals that are toxic, physiological metabolites, and insecticides). Infertility can be caused during cancer treatment either permanently or temporarily. Extreme body mass, drugs, alcohol, cigarette, late work hours, stress, unhealthy lifestyle all have negative impact on fertility. **Homan et al. [17]** Along with the more prevalent causes

of male infertility, such as varicocele, cryptorchidism, obstructive tumors, cystic fibrosis, trauma, and tumors, oxidative stress has been identified as a novel and important factor. **Makker et al. [24]** The characteristics of the sperm's state of life may be affected by this, and ROS-induced DNA damage may also result. **Cho et al. [12]**



Fig.1.2 Various causes of Infertility

### 1.3 Stress and Male Infertility

One of the many factors associated with the male component of infertility is oxidative stress (OS), and it has been demonstrated to have an effect on men's fertility. **Agarwal et al. [3]**

With excessive ROS free radical production, which results in an oxygen anomaly where free radicals are needed for cell function but, at high concentrations, may interfere with crucial chemical reactions, oxidative stress is a condition

where the cell's antioxidant defense system is underrated. **Valko et al. [34]** Spermatozoa experience the "oxygen-paradox" frequently, much like practically all other cells that consume oxygen. **Sies H. et al. [31]** Reactive oxygen species (ROS) are created and act as free radicals. A few examples of ROS include the OH-ion, superoxide, H<sub>2</sub>O<sub>2</sub> ion, peroxy radical, and hypochlorite ions. High quantities of these may put sperm survival at risk. **Agarwal et al. [2]**

#### 1.4 Pathophysiology of oxidative stress induced infertility

The production of 4-hydroxynonenal and malondialdehyde (MDA) as a result of lipid membrane peroxidation and DNA damage in both the nuclear and mitochondrial compartments is accompanied by the reduction of intracellular energy (ATP) stores, which is the primary cause of oxidative stress-induced semen quality reduction. **Aitken et al. [4], De Lamirande et al. [13]**

Sperm cells with damaged DNA have very limited capacity for spontaneous conception. A considerable degree of DNA damage in male spermatozoa has been associated to unfavorable health outcomes such as infertility, recurrent pregnancy losses, childhood mortality, and extremely high rates of deaths in children from inherited illnesses, challenging circumstances, and pediatric cancers. **Aitken et al. [5]**

Since DNA mutations in the male's germline are a significant risk factor for serious medical conditions such as deprived fertilization, late growth in the embryo, loss of pregnancy, and a greater chance of death and morbidity in the

progeny, the development of appropriate treatment methods that aid in the evaluation of DNA damage in spermatozoa is too much needed for the treatment of male infertility disorders. As a result of concurrent damage to the protein and lipid in the male cell plasma membrane, which affects both the fluidity as well as the permeability of the cell membrane, oxidative stress compromises the functioning of sperm by compromising DNA integrity. **Aitken et al. [6]**

In today's modern and constantly changing world, stress is a crucial component of every culture, and infertility is upsetting due to societal expectations, examination, being diagnosed, therapies, blunders, and even the financial costs that come along with it. **Anderson et al. [7]**

Different persons and situations will interpret stress differently. Hans Selye has defined that stress is the human body's general response to any stimulus. **Fink et al. [15]**

By reducing testosterone pulses, stress can alter sperm characteristics. Glucocorticoids are produced in large quantities as a result of stress, and this can cause Leydig cells, Sertoli cells, and germ cells to die. The loss of these cells may

lead to a reduction in the quantity and quality of sperm by reducing the levels of the hormones LH and testosterone.

#### **Ilacqua et al. [18]**

One of the possible causes of the suppression of male reproductive function caused by depression in the hypothalamic-pituitary-testicular axis is stressor activation of the hypothalamic-pituitary-adrenal axis. **Retana et al. [30]**

#### **1.5 Factor Causing Oxidative Stress induced Male Infertility**

Smoking, binge drinking, eating foods full of saturated fat and proteins, being inactive, feeling stressed out, being overweight, and a male's older age (> 40 years of age) all increase the likelihood of experiencing infertility. Furthermore, severe temperatures may be too high or too cold, plasticizing substances, contaminants (particularly transition metals like (Cd), Pb, Fe, and Cu), chemotherapy treatments, toxic chemicals (acrylamide, endosulfan, bisphenol A, and phthalates), microwaves, blood infection, and varicocele may all increase the amount of oxidative stress in sperm cells. **K. Tremellen [32], Kumar et al. [21]**

Nicotine consumption, drinking alcohol, as well as living nearby dangerous chemicals, may all be connected to the risk of sperm quality deterioration and higher levels of oxidative stress and DNA damage.

Many antioxidants, such as Tocopherol, vitamins C, glutathione, albumin, and SOD, are available as nutritional supplements. Some of these medications increase the number of sperm and their mobility. However, few have been proved to improve damaged DNA in sperm at therapeutic doses. **Walczak-**

#### **Jedrzejowska et al. [36]**

#### **1.6 Treatment Approaches of Male Infertility**

Most of documented explanations for male infertility have a therapy target of maintaining the reproductive axis so as to raise testicular testosterone. The current therapeutic techniques are as follows:-

##### **1.6.1 Hormonal Therapy**

1. (GnRH):- Pulsatile discharge Injecting GnRH is a successful treatment for infertile males who lack the hormone and will enhance spermatogenesis. The most effective dose for GnRH pulsatile release is 5-20 g administered via S.C.

injection every one to two hours. **Happ et al. [16]**

2. Gonadotropins:- Gonadotropins are the basis for treating infertility in men with hormonal deficiency. We only administer gonadotropins via the SC route in doses ranging from 75 to 150 units of FSH or hMG two to three times per week and 1,500 to 2,500 IU of hCG twice per week. The duration of this treatment may range from 6 to 24 months, or it may go longer, and it will continue until sperm appears in the ejaculate or a pregnancy develops. Spermatogenesis is triggered by gonadotropins. **Burgués et al. [9]**

**1.6.2 Dopamine agonist :** High Prl levels in hypogonadically infertile men reduce the production of GnRH on a continuous basis. Agonists of dopamine are also recommended for the management of male infertility. Cabergoline and Bromocriptine are prescribed. **Webster et al. [37]**

### **1.6.3 Androgen Therapy**

Testosterone therapy is used to treat male infertility. For men with hypogonadism, take one 40 mg capsule of testosterone undecanoate daily. Testosterone therapy is

used to treat male infertility. For men with hypogonadism, take one 40 mg capsule of testosterone undecanoate daily. For male infertility, oligozoospermia, and androgen deficiency, a 25 mg pill is taken one to three times daily. **K.D. Tripathi [33]**

### **1.6.4 Aromatase inhibitor (AI) therapy**

AIs, such as (Anastrozole 1 mg or Letrozole 2.5 mg tab daily), increase testosterone levels while lowering oestrogen levels and obstruct the peripheral metabolism of testosterone. **Bharti et al. [8]** The Leydig cells' aromatase activity may contribute to the conversion of testosterone (T) to estradiol (E) and the impairment of sperm parameters, according to aromatase inhibition (AI) activity. **Raman et al. [28]**

### **1.6.5 Selective estrogen receptor modulators (SERMs)**

This refers to a particular class of chemical that can either bind to or inhibit the oestrogen receptor. In hypogonadal males, clomiphene can significantly improve serum testosterone levels. **Katz et al. [20]** There is evidence that taking 20 mg of tamoxifen or 60 mg of toremifene +

raloxifene daily would improve sperm quality. **Farmakiotis et al. [14]**

### 1.6.6 In- Vitro Fertilization

In-vitro fertilization (IVF) is the most popular medical procedure used to treat infertility. In vitro fertilization (IVF) entails the use of fertility drugs to increase

## 2. MATERIALS AND METHODS

*Brassica oleracea* var. *italica* commonly known as Broccoli. Vitamins, glucosinolates, and phenolic compounds, among others, are abundant in broccoli buds and help to maintain good health. It is essential to have naturally occurring antioxidants such flavonoids and phenolic compounds. Additionally, it includes DPPH (2,2-diphenyl-1-picrylhydrazyl) and vitamin C. **Le et al. [22]** Additionally, it has a lot of folic acid.

In addition to having anti-cancer, anti-diabetic, and neurodegenerative disease prevention qualities, *Brassica oleracea* var. *italica* also has effects on asthma and

egg production, removal of the infertile female ovary's egg(s), and combining the removed egg(s) with sperm in a culture chamber in a test tube. **Lundborg et al. [23]** If an egg fertilizes, it is placed back into the infertile woman's uterus; the success of the transfer is shown by the egg adhering to the uterine walls.

exhibits antioxidant properties. **Chandini Ravikumar [29]**

### 2.1 Extraction of plant material

The broccoli florets, *Brassica oleracea* var. *italica*, were divided into bits, dried under the shade, and then ground with a mortar and pestle. Using a laboratory grinder, the crushed plant material was coarsely ground. Using a laboratory grinder, the crushed plant material was coarsely ground. Then a thimble was filled with 1000 g of *Brassica oleracea* var. *italica* (broccoli) powder, and the soxhlet was sealed. They were then then effectively extracted using the hot extraction method at 60 degrees Celsius and 70% ethanol. The device was left running for 72 hours to allow the colored solvent to appear in the syphon for the preliminary crude

extracts of the compounds. The extracted extracts were placed in a water bath to air out once the solvent had fully evaporated, and then they were placed in an airtight container and kept in the refrigerator. The result was 52.3 g of solid residual with a yield of 5.23 percent by volume.

Various phytochemical and physiochemical analysis were performed to demonstrate the presence of antioxidants.

## 2.2 PHARMACOLOGICAL EVALUATION

Male Wistar rats were purchased from Chakraborty Enterprises, Kolkata, West Bengal (Reg No. 1443/PO/BT/s/11/CPCSEA). Lab rats (250-300g) were housed in a room with a constant temperature of 24.2 °C, a relative humidity of 60 5%, and a cycle of 12 h of light and 12 h of darkness. The rats got a week to get acclimated to the lab setting before the experiment and were housed in air-conditioned animal houses with free pelleted food and running water. **Chidrawar et al. [11]** The O.E.C.D recommendations are followed for acute oral toxicity.

## 2.3 Experimental Induction of Stress Induced Infertility

Rats underwent the Forced Swimming Test stress model every day from 9:00 am to 10:00 am for 15 days. **Nayanatara et al. [26]**

Animals were submerged in a glass tank with dimensions of L 100 cm, W 40 cm, and depth 60 cm, which was kept at a temperature of 36 C. In order to prevent the rats from taking a nap by resting their tails on the tank's bottom, the water's depth was set at 35 cm. Whenever an animal fails to rise to the water's surface to breathe. It was known that they had been put out in less than 7 seconds. At this moment, the animals were removed from the tank. **Zhang et al. [39]**

## 2.4 Experimental Design and Procedure

For this experiment, 30 mature male Wistar rats weighing 120-180g on average were used. The rats were split up into 5 groups of 6 each.

**Group1:** Negative control animals which received only Distilled water.

**Group 2:** Positive control group (given Stress by Forced Swimming Test).



**Group 3:** Standard group infertility induced by stress treated with standard drug Sildenafil 5mg/kg orally by gavage.

**Group 4:** Stress + Low dose *Brassica oleracea* var. *italica* (Broccoli) extract (250mg/kg/day).

**Group 5:** Stress + High dose *Brassica oleracea* var. *italica* (Broccoli) (500mg/kg/day).

## 2.5 BIOCHEMICAL PARAMETER

### 2.5.1 Oxidative stress status in serum, lipid profile for testes cholesterol

Each animal was weighed and sacrificed by cervical dislocation on the 16th day, 24 hours after the final injection. Heart punctures were used to collect blood samples. Serum was used to determine the lipid profile (LDL, HDL, and VLDL), oxidative stress parameters (level of SOD, CAT, MDA, and glutathione), and hormonal tests for testosterone.

Vijayprasad [35]

### 2.5.2 Measurement of Sperm parameters and Hormonal Assay for Testosterone

## 3. RESULTS

In order to examine the sperm parameters, the abdomen of rats was dissected, and the tail of each testis' epididymis was severed. Sperm characteristics like motility, viability, and count are measured. Raeeszadeh et al. [27]

### 2.5.3 Measurement of Body and Organ weight

The testes, prostate, and testicular index were weighed on the sixteenth day following dissection.

## 2.6 HISTOPATHOLOGICAL STUDY

The testes were removed from the animal under investigation after its death on the sixteenth day of the study and stored in an airtight container with formalin (10% v/v) at a temperature below 37°C while being incubated in a clean environment for histopathology evaluation. In the end, a light microscope was used to examine testicular histopathological lesions.

The histopathology was done at United Diagnostic and Research, Prayagraj.

## 3.1 PHARMACOLOGICAL EVALUATION

### 3.1.1 Measurement of Sperm Parameter and Hormonal Assay for Testosterone

The negative control groups were not provided any drugs or stressors; they were merely given distilled water. Infertility was caused in the positive control group by a forced swim test, and stress was introduced in the other groups using the same technique. Sildenafil 5mg/kg was administered to the standard group. The two more groups of animals are handled with the BOVI (*Brassica oleracea* var. *italica*) extract in both low and high doses. The 15-day experimental trial was conducted. BOVI crude extract is provided. sperm count increases dose-dependently when taken orally at doses of 250 mg/kg and 500 mg/kg.

Multiple sperm metrics (count, motility, and viability), as well as the testosterone level, significantly decreased in the positive control group. Remarkably enhanced sperm parameters and testosterone levels were seen in the Sildenafil-treated standard group. BOVI extract considerably increases testosterone levels and sperm parameters at low and high doses.

Sperm count is recorded as  $212.33 \pm 42.64$  in low dose 250 mg/kg group and  $226.83 \pm 38.38$  in High Dose 500 mg/kg compared to Positive control group  $147.52 \pm 31.24$ . Along with Sperm count, testosterone level also recorded as  $1.17 \pm 0.15$  in low dose BOVI group and  $1.75 \pm 0.19$  in High Dose BOVI group compared to positive group  $0.97 \pm 0.19$ .

**Table 3.1 Effect of BOVI 250mg/kg and BOVI 500mg/kg on Sperm parameters and Testosterone**

Groups	Sperm count	Sperm motility %	Sperm viability %	Testosterone
Negative control	258.16±32.02	91.33±8.64	82.16±4.44	2.24±0.39
Positive control	147.52±31.24 <sup>a</sup>	67.66±3.61 <sup>a</sup>	67.16±4.07 <sup>a</sup>	0.97±0.19 <sup>a</sup>

STD Sildenafil 5 mg/kg	260.16±31.97**	93.16±9.82**	81.83±5.52**	3.44±0.51**
BOVI 250 mg/kg	212.33±42.64**	69.66±2.81**	68.33±3.55**	1.17±0.15**
BOVI 500 mg/kg	226.83±38.38**	87.33±8.23**	77.33±6.21**	1.75±0.19**

The data depicts as mean±SD of 6 animals from each group  $p < 0.05^a$  in comparison of positive group with negative group and  $p < 0.001^{**}$  show significant difference as compared to BOVI treated group with positive.

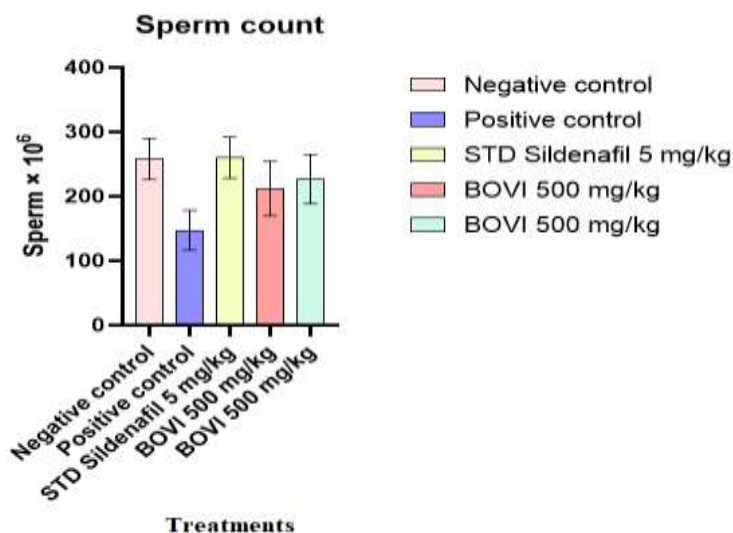
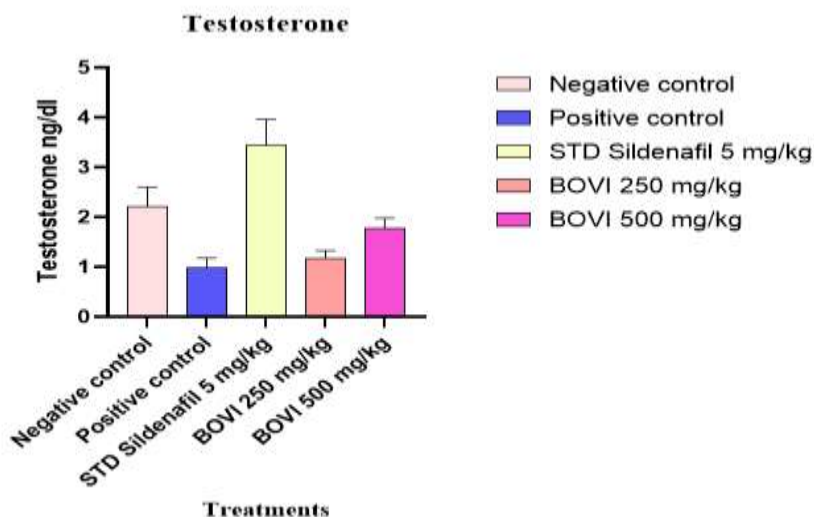


Fig. 3.1 Representation of Sperm count in different groups



**Fig 3.2 Representation of testosterone hormone in different groups**

### 3.1.2 Measurement of Oxidative stress marker

In positive control group Oxidative stress markers (SOD, CAT and MDA) rises significantly. Standard Sildenafil treated group shows decreased oxidative stress markers i.e.,  $21.92 \pm 3.11$  SOD,  $19.49 \pm 0.72$  CAT, and  $2.76 \pm 0.84$  MDA compared to Positive control group i.e.,  $33.75 \pm 10.59$  SOD,  $32.22 \pm 1.74$  CAT,  $6.63 \pm 1.14$  MDA.

Low dose 250mg/kg & High dose 500mg/kg of BOVI extract shows significant decrease in oxidative stress markers (SOD, CAT and MDA) compared to positive groups.

The data depicts as mean $\pm$ SD of 6 animals from each group  $p < 0.01^a$  in comparison of positive group with negative group and  $p < 0.001^{##}$  show significant difference as compared to BOVI treated group with positive.

**Table 3.2 Effect of BOVI 250mg/kg and BOVI 500mg/kg on Oxidative Stress Markers**

Group	SOD (U/ml)	CAT (U/ml)	MDA (nm/ $\mu$ L)
Negative control	$13.21 \pm 1.31$	$17.24 \pm 2.30$	$2.68 \pm 0.23$
Positive control	$33.75 \pm 10.59^a$	$32.22 \pm 1.74^a$	$6.63 \pm 1.14^a$

STD Sildenafil 5 mg/kg	21.92±3.11 <sup>##</sup>	19.49±0.72 <sup>##</sup>	2.76±0.84 <sup>##</sup>
BOVI 250 mg/kg	27.55±3.42 <sup>##</sup>	24.87±1.73 <sup>##</sup>	5.46±1.10 <sup>##</sup>
BOVI 500 mg/kg	23.94±3.30 <sup>##</sup>	20.26±1.02 <sup>##</sup>	3.58±0.84 <sup>##</sup>

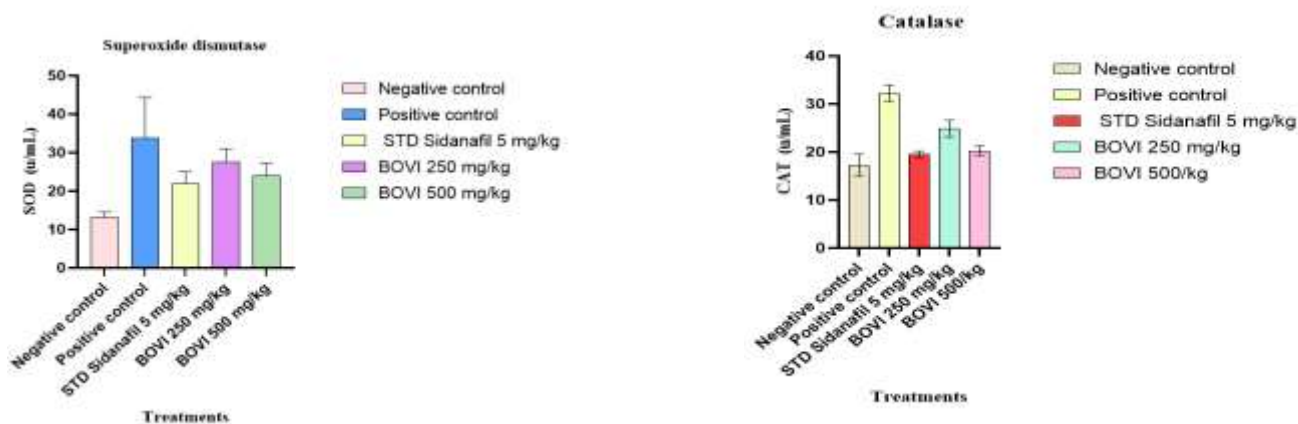


Fig. 3.3 Graphical representation of SOD in different groups treatment groups

Fig 3.4 Graphical representation of CAT in different

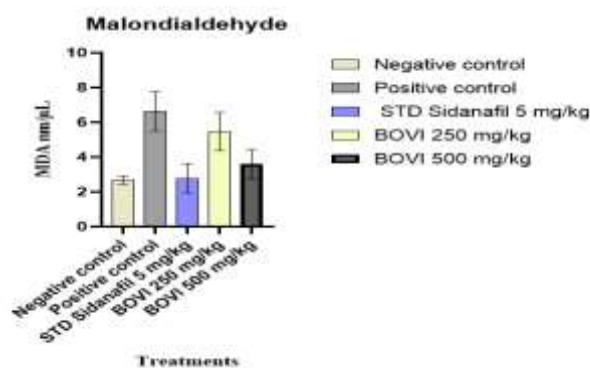


Fig 3.5 Graphical representation of MDA in different groups

### 3.1.3 Body and Reproductive Organ Weight

In each group, the ultimate body weight (measured on day 16) is lower than the initial weight (measured on day 1).

Significant changes in body weight are seen in Positive control group. Testicular weight also decreased significantly in

positive group. BOVI extract treated groups shows gradual increase in reproductive organ weights.

**Table 3.3 Effect of BOVI 250mg/kg and BOVI 500mg/kg on Body and Reproductive organ weight**

Groups	Body weight		Testis weight		Prostate weight	Testicular Index %
	Initial	Final	Left	Right		
Negative control	127.16±4.75	128.52±3.14	1.081±0.056	1.124±0.056	0.128±0.006	0.841±0.047
Positive control	149.51±4.72 <sup>a</sup>	135.546.05 <sup>a</sup>	0.903±0.104 <sup>a</sup>	0.971±0.121 <sup>a</sup>	0.100±0.004 <sup>a</sup>	0.669±0.101 <sup>a</sup>
STD Sildenafil 5 mg/kg	136.83±3.97 <sup>***</sup>	135.33±4.13 <sup>***</sup>	1.066±0.059 <sup>***</sup>	1.169±0.052 <sup>***</sup>	0.126±0.003 <sup>***</sup>	0.787±0.047 <sup>***</sup>
BOVI 250 mg/kg	139.16±8.51 <sup>***</sup>	135.51±8.14 <sup>***</sup>	0.966±0.084 <sup>***</sup>	1.076±0.051 <sup>***</sup>	0.117±0.023 <sup>***</sup>	0.691±0.061 <sup>***</sup>
BOVI 500 mg/kg	142.28±3.03 <sup>***</sup>	140.33±3.61 <sup>***</sup>	1.033±0.059 <sup>***</sup>	1.169±0.226 <sup>***</sup>	0.119±0.007 <sup>***</sup>	0.766±0.079 <sup>***</sup>

The data depicts as mean±SD of 6 animals from each group p<0.05<sup>a</sup> in comparison of positive group with negative group and p<0.001<sup>\*\*\*</sup> show significant difference as compared to BOVI treated group with positive.

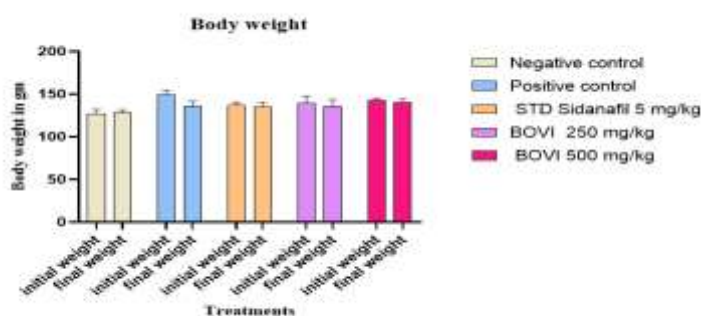


Fig 3.6 Representation of initial & final body weight in different groups

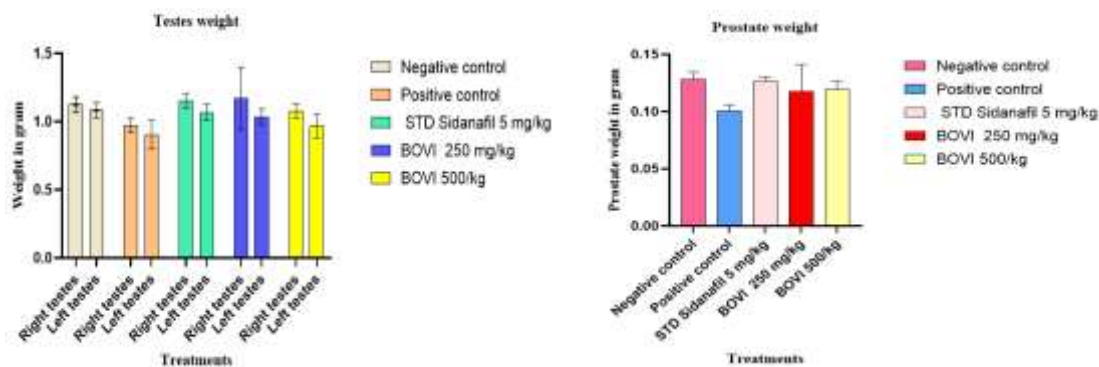


Fig 3.7Representation of right and left testes weight in diff group

Fig 3.8 Representation of weight of prostate in diff. group

### 3.1.4 Measurement of Lipid Profile

Total cholesterol, triglycerides and LDL increases significantly in Positive control group. BOVI extract treatment given groups decreases the LDL, total cholesterol and triglyceride level significantly compared to positive control group.

Table 3.4 Effect of BOVI 250mg/kg and BOVI 500mg/kg on Lipid Profile

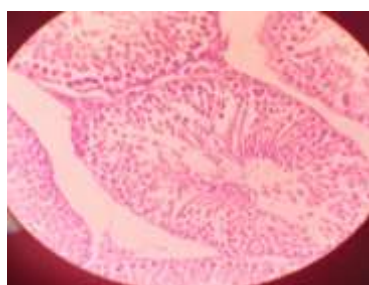
Groups	Total cholesterol	Triglyceride	HDL	VLDL	LDL
Negative control	105.46±1.57	67.12±2.64	39.45±1.38	21.38±1.19	74.16±2.38
Positive control	201.32±2.27*	90.47±0.38*	41.60±0.60*	34.44±0.46*	147.30±1.56*
STD Sildenafil 15 mg/kg	196.59±3.92#	70.08±0.66#	40.94±0.57#	26.67±0.95#	141.200±0.95#
BOVI 250 mg/kg	197.69±3.92#	80.89±0.78#	41.79±0.75#	27.23±1.09#	142.80±1.05##

BOVI 500 mg/kg	196.91±3.57 <sup>#</sup>	75.41±1.73 <sup>#</sup>	41.02±0.73 <sup>#</sup>	26.87±0.73 <sup>#</sup>	141.38±0.48 <sup>##</sup>

The data depicts as mean±SD of 6 animals from each group  $p < 0.05^*$  in comparison of positive group with negative group and  $p < 0.001^{##}$  show significant difference as compared to BOVI treated group with positive.

### 3.2 HISTOPATHOLOGICAL ANALYSIS

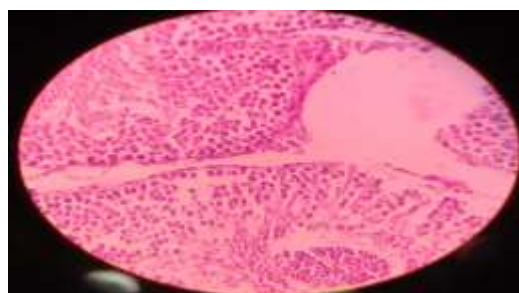
Fig. 4.9 Histological sections of testes from different groups from (a) to (e) are shown. (a) Negative control group, (b) Positive control (stress only), (c) Standard group (Sildenafil 5mg/kg), (d) Low Dose 250mg/kg, (e) High dose 500mg/kg. Black pointer depicts Spermatogonia, Blue pointer depicts primary spermatocytes and green pointer depicts spermatids.



(a) Normal Control

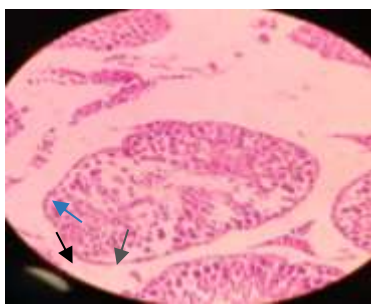


(b) Negative control

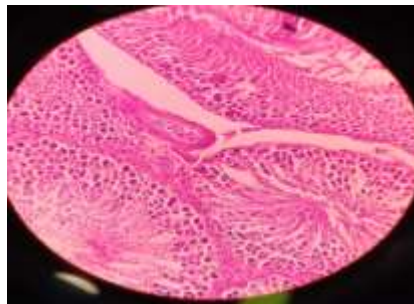


(c) Positive Control





(d) Low Dose 250 mg/kg



(e) High Dose 500 mg/kg

#### 4. DISCUSSION

Stress and infertility are correlated with one another. Infertility can lead to stress, and stress can lead to infertility. Treatment for infertility is a demanding process in and of itself. This may be the result of peer and social pressure. Infertility is one of the effects of stress that has received recent attention. Male factor infertility is more common but less discussed. Additionally, understanding oxidative stress offers several therapy modalities that are tested to enhance infertility. **Makker et al. [24]** By evaluating the weight of the body and the reproductive organs, as well as biochemical and histological data, the Forced Swim Test model is used to detect stress-induced infertility in male rats. By enhancing the quality of sperm testing and sperm parameters, many antioxidants have demonstrated their ability to alleviate

oxidative stress and the infertility it causes.

The Forced Swim test model, which is now the most widely used model for creating stress, was used to assess whether the ethanolic extract of the inflorescence of *Brassica oleracea* var. *italica* caused stress-induced infertility in male wistar rats. **Nayanatara et al. [25]** In our research, stress greatly lowers testosterone levels and sperm counts in rats, but these levels are significantly boosted when the rats are given *Brassica oleracea* var. *italica* (broccoli) extract. While the High Dose (500 mg/kg) of *Brassica oleracea* var. *italica* significantly increases sperm count, testosterone level, and other biochemical markers, the Low Dose (250 mg/kg) has no discernible effects. The impact of *Brassica oleracea* var. *italica* is therefore dose-dependent.

#### 5. CONCLUSION

In this day of cutting-edge technology, stress-related infertility is the most prevalent illness. which the male component is mainly responsible for. The overproduction of ROS brought on by oxidative stress damages sperm and results in lipid peroxidation, which lowers sperm quality and quantity and alters testosterone levels.

Finally, we can state with certainty that alkaloids, carbohydrates, glycosides, phenols, flavanoids, steroids, and proteins are among the phytoconstituents present in Brassica oleracea var. italica extract. The presence of phenol and flavonoids supports the plant's ability to act as an antioxidant.

Brassica oleracea var. italica extract is used to treat stress-related infertility in doses of 250 mg/kg and 500 mg/kg, respectively. increased testosterone levels, lowered body weight, improved antioxidant enzymes (SOD, CAT, and MDA), improved lipid profile, and increased sperm count and quality of other sperm parameters.

Group treated with a high dose exhibits notable alterations. Therefore, the action

of Brassica oleracea var. italica is dose-dependent.

We can draw the conclusion that the data point to Brassica oleracea var. italica (broccoli) as a potential novel medication candidate or nutraceutical for the treatment of stress-related infertility.

## 6. REFERENCE

1. Agarwal A., Baskaran S., Parekh N., Cho C. L., Henkel R., Vij S., ... & Shah R. (2021). Male infertility. *The Lancet*, 397(10271), 319-333.
2. Agarwal Ashok, Kartikeya Makker, and Rakesh Sharma. "Clinical relevance of oxidative stress in male factor infertility: an update." *American journal of reproductive immunology* 59, no. 1 (2008): 2-11
3. Aiswarya S. P. "A study on causes of female infertility." PhD diss., Sree Mookambika Institute of Medical Sciences, Kulasekharam, 2018.
4. Aitken R. J. Human spermatozoa: revelations on the road to conception. *F1000Prime Rep.* 5, 39 (2013).
5. Aitken R. J., De Iuliis G. N. & McLachlan R. I. Biological and clinical significance of DNA damage in the male germ line. *Int. J. Androl.* 32, 46–56 (2009).
6. Aitken RJ, Gibb Z, Mitchell LA, Lambourne SR, Connaughton HS, De Iuliis GN. Sperm motility is lost in vitro as a consequence of mitochondrial free radical production and the generation of electrophilic aldehydes but can be significantly rescued by the presence of nucleophilic thiols. *Biol Reprod.* 2012 Nov 8;87(5):110. doi: 10.1095/biolreprod.112.102020. PMID: 22933515.
7. Anderson K, Niesenblat V, Norman R. Lifestyle factors in people seeking infertility treatment. A review. *Aust N Z J Obstet Gynaecol.* 2010;50:8–20.
8. Bharti S, Misro MM, Rai U. Clomiphene citrate potentiates the adverse effects of estrogen on rat testis and down-regulates the expression of steroidogenic enzyme genes. *Fertil Steril* 2013;99:140-8.
9. Burgués S, Calderón MD. Subcutaneous selfadministration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotropic hypogonadism. Spanish Collaborative Group on Male Hypogonadotropic Hypogonadism. *Hum Reprod* 1997;12:980-6.
10. Cattapan A, Baylis F. Frozen in perpetuity: 'abandoned embryos' in Canada. *Reprod Biomed Soc Online.* 2016 May 12;1(2):104-112. doi: 10.1016/j.rbms.2016.04.002. PMID: 29911191; PMCID: PMC6001352.

11. Chidrawar V. R., Chitme H. R., Patel K. N., Patel N. J., Racharla V. R., Dhoraji N. C., & Vadalia, K. R. (2011). Effects of *Cynodon dactylon* on stress-induced infertility in male rats. *Journal of Young Pharmacists*, 3(1), 26-35.
12. Cho CL, Agarwal A, Majzoub A, Esteves SC. Clinical utility of sperm DNA fragmentation testing: concise practice recommendations. *Transl Androl Urol*. 2017;6:S366–73
13. De Lamirande E. & Gagnon C. Reactive oxygen species and human spermatozoa: I. Effects on the motility of intact spermatozoa and on sperm axonemes. *J. Androl*. **13**, 368–368 (1992).
14. Farmakiotis D, Farmakis C, Rouso D, et al. The beneficial effects of toremifene administration on the hypothalamic-pituitary-testicular axis and sperm parameters in men with idiopathic oligozoospermia. *Fertil Steril* 2007;88:847-53.
15. Fink G. "Stress: Concepts, Definition, and History George." *Neuroscience and Biobehavioral Psychology*. Amsterdam, The Netherlands: Elsevier (2017).
16. Happ J, Ditscheid W, Krause U. Pulsatile gonadotropin-releasing hormone therapy in male patients with Kallmann's syndrome or constitutional delay of puberty. *Fertil Steril* 1985;43:599-608.
17. Homan G.F., Davies M., Norman R., 2007. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Hum. Reprod. Update* 13, 209–223.
18. Ilacqua A., Izzo G., Emerenziani G. P., Baldari C., & Aversa A. (2018). Lifestyle and fertility: the influence of stress and quality of life on male fertility. *Reproductive Biology and Endocrinology*, 16, 1-11.
19. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. *Hum Reprod Update*. 2015 Jul-Aug;21(4):411-26. doi: 10.1093/humupd/dmv016. Epub 2015 Mar 22. PMID: 25801630.
20. Katz DJ, Nabulsi O, Tal R, et al. Outcomes of clomiphene citrate treatment in young hypogonadal men. *BJU Int* 2012;110:573-8.
21. Kumar S., Murarka S., Mishra V. & Gautam A. Environmental & lifestyle factors in deterioration of male

- reproductive health. *Indian J. Med. Res.* **140**, 29 (2014).
22. Le T. N., Luong H. Q., Li H. P., Chiu C. H., & Hsieh P. C. (2019). Broccoli (*Brassica oleracea* L. var. *italica*) sprouts as the potential food source for bioactive properties: A comprehensive study on in vitro disease models. *Foods*, 8(11), 532
23. Lundborg P., Plug E., & Rasmussen A. W. (2014). Fertility effects on femapa labor supply: IV evidence from IVF treatments.
24. Makker K., Agarwal A., & Sharma R. (2009). Oxidative stress & male infertility. *Indian Journal of Medical Research*, 129(4), 357
25. Nayanatara AK, Vinodini NA, Ahemed B, Ramaswamy CR, Shabarianth Ramesh Bhat. Role of ascorbic acid in monosodium glutamate mediated effect on testicular weight, sperm morphology and sperm count, in rat testis. *Journal of Chinese Clinical Medicine*. 2008;3(1):1-5.
26. Nayanatara A. K., Nagaraja, H. S., & Anupama, B. K. (2005). The effect of repeated swimming stress on organ weights and lipid peroxidation in rats. *Thai J Pharm Sci*, 18(1), 3-9.
27. Raeeszadeh M., & Akbari A. (2021). The effects of broccoli and caraway extracts on serum oxidative markers, testicular structure and function, and sperm quality before and after sperm cryopreservation. *Cryobiology*, 99, 11-19.
28. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. *J Urol* 2002;167:624-9.
29. Ravikumar C. (2015). Therapeutic potential of *Brassica oleracea* (broccoli)—a review. *Int J Drug Dev Res*, 7(7), 9-10.
30. Retana MS, Salazar ED, Velazquez M. Effect of acute and chronic stress on masculine sexual behavior in the rats. *Psychoneuropharmacology* 1996;21:39-50.
31. Sies H. Strategies of antioxidant defense. *Eur J Biochem* 1993; 275: 213-9.
32. Tremellen K. Oxidative stress and male infertility — a clinical perspective. *Hum. Reprod. Update* **14**, 243–258 (2008).
33. Tripathi K. D. *Essentials of medical pharmacology*. JP Medical Ltd, 2013.
34. Valko M. *et al.* Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **39**, 44–84 (2007).

35. Vijayprasad S., Ghongane B. B., & Nayak B. B. (2014). Effect of vitamin C on male fertility in rats subjected to forced swimming stress. *Journal of clinical and diagnostic research: JCDR*, 8(7), HC05.
36. Walczak-Jedrzejowska R., Wolski J. K. & Slowikowska- Hilczer J. The role of oxidative stress and antioxidants in male fertility. *Cent. European J. Urol.* **66**, 60–67 (2013).
37. Webster J, Piscitelli G, Polli A, et al. Dose-dependent suppression of serum prolactin by cabergoline in hyperprolactinaemia: a placebo controlled, double blind, multicentre study. European Multicentre Cabergoline Dose-finding Study Group. *Clin Endocrinol (Oxf)* 1992;37:534-41.
38. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The international glossary on infertility and fertility care, 2017. *Fertil Steril* 2017; 108: 393–406.
39. Zhang Dao-rong, Wan Yu, Zu Jing, Zhan Jian-guo, Li Lei and Bai Jian-qing. *Morinda officinalis* How enhances exercise endurance and possesses protective effects against oxidative stress of the rats after exercise. *African Journal of Microbiology Research*.2010;4(15): 1609-15.