# PROTECTIVE ROLE OF *NAGA PARPAM* –A ZINC BASED HERBO-MINERAL FORMULATION AGAINST MONOCROTOPHOS INDUCED FERTILITY CHANGES IN MALE WISTAR RATS

**A Dissertation submitted to**

**THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY CHENNAI - 600 032**

**In partial fulfillment of the requirements for the award of the Degree of**

**MASTER OF PHARMACY**

**IN**

**BRANCH-IX PHARMACOLOGY**

**Submitted by BOOBESH. H**

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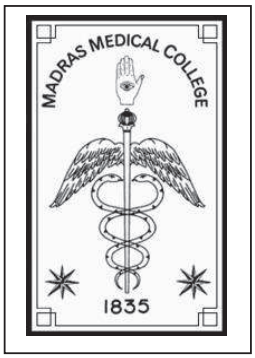


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## CERTIFICATE

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**Date :**
**Place : Chennai - 03**

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# CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 4-HNE | 4-hydroxy-nonenal |
| AI | Aromatase inhibitor |
| ATPase | Adenosine triphosphatase |
| CASA | Computer assisted semen analysis |
| CAT | Catalase |
| CI | Coital infertility |
| CMC | Carboxy methyl cellulose |
| CoQ10 | Co enzyme Q10 |
| DDT | Dichloro Diphenyl Trichloroethane |
| DI | Donor insemination |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl hydrate |
| ECLIA | Electro chemiluminescence immunoassay |
| ED | Endocrine disruptors |
| EDC | Endocrine disrupting chemicals |
| EPA | Environmental Protection Agency |
| GABA | Gamma amino butyric acid |
| GnRH | Gonadotropin-releasing hormone |
| GPx | Glutathione peroxidase |
| $H_2O_2$ | Hydrogen peroxide |
| HCG | Human chorionic gonadotrophin |
| HMG | Human Menopausal Gonadotrophin |
| HSV | Herpes simplex virus |
| ICMI | Intra cytoplasmic morphologically selected sperm injection |

| | |
|---|---|
| IGR | Insect growth regulators |
| IMPCOPS | Indian Medical Practitioner's Co-Operative Society |
| IUI | Intrauterine insemination |
| LDL | Low-density lipoproteins |
| LPO | Lipid peroxidation |
| MCP | Monocrotophos |
| MDA | Malondialdehyde |
| MOSI | Male oxidative stress infertility |
| MTT | 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide |
| NOI | Non-obstructive Infertility |
| NP | Naga Parpam |
| OI | Obstructive Infertility |
| PICSI | Physiological Intra cytoplasmic sperm injection |
| PTTH | Prothoracicotropic hormone |
| ReE | Retrograde ejaculation |
| ROS | Reactive oxygen species |
| SAS | Free radical scavenging activity |
| SCO | Sertoli cell only |
| SERM | Selective Estrogen Receptor Modulators |
| SOD | Superoxide dismutase |
| SSR | Surgical sperm recovery |
| STD | Sexually transmitted diseases |
| TAP | Total antioxidant power |
| TBARS | Thiobarbituric acid reactive substances |

| | |
|---|---|
| TBO | Testicular biopsy |
| TEPP | Tetra Ethyl Pyrophosphate |
| TF | Testicular failure |
| UCr | Upper Cryptorchidism |
| UU | Ureaplasma urealyticum |
| VLDL | Very low-density lipoproteins |
| WHO | World Health Organization |
| ZnO | Zinc oxide |

# INTRODUCTION

# 1. INTRODUCTION

The upsurge in male infertility over the last two decades, possibly due to environmental exposure, has raised significant interest, particularly boosted by reports from fertility clinics, which showed that chronic diseases and hereditary or other medical conditions might only partially explain current incidence of male infertility. Both environmental and occupational settings may have a significant role in exposure to complex mixtures of endocrine disruptors (ED), which play a major role in fertility disorders. [(1)]

Male factor infertility is said to be present when a couple fails to achieve pregnancy after one year of unprotected coitus and a problem is identified in the male partner. [(2)]

It affects 10–15 % of all couples and varies between countries and geographic regions. Men and women contribute equally to the problem, and often more than one infertility factor is involved [(3)]

Occupational exposure occurs while mixing, loading, spraying and assessment of the pesticides. There are different types of occupational hazards affecting reproductive organs in both males and females.[(4)] But, male reproductive activity is highly sensitive to many man-made chemicals and physical agents produced by agricultural and industrial activities.[(5)]

A pesticide is defined as "any substance or combination of sub-stances used to prevent or eradicate unwanted insects including vectors of diseases in human-beings and animals, weeds, fungi or animals in order to enhance food production and help production processing, storage, transport, or marketing of the food and agricultural commodities"[(6)]

Pesticides act as endocrine disrupting chemicals (EDCs). The EDC is defined by US environmental protection agency (EPA) as "an exogenous agent that is potentially capable of synthesis, secretion, transport, binding, action, or elimination of the natural hormones responsible for the maintenance of homeostasis, reproduction, and developmental processes in the body" [(7)]

**Common insecticides are usually classified on the basis of their chemical nature** [(8)(9)]

1. **Arsenical insecticides** based on inorganic arsenite, e.g., Calcium arsenate, potassium/sodium arsenite, Copper acetoarsenite / lead arsenate.
2. **Organochlorine insecticides**, e.g., DDT, Lindane, Pentachlorophenol.
3. **Organophosphorus insecticides**, e.g., Dichorvos, TEPP, Malathion, Chlorpyrifos etc.

4. **Carbamate insecticides**, e.g., Carbaryl, Carbofuran, Methomyl, Propoxur, etc.

5. **Oxadiazine insecticides**, e.g., Indoxacarb.

6. **Pyrrole insecticides**, e.g., Chlorfenapyr.

7. **Pyrazole insecticides**, e.g., Chlorantraniliprole, Dimetilan, Tolfenpyrad.

**8. Phenylpyrazole insecticides**, e.g., Acetoprole, Fipronil, Pyraclofos,pyriprole.

9. **Pyrethroid insecticides**, e.g., Allethrin, Barthrin, Cypermethrin, Deltamethrin, Fenvalerate, Permethrin, Resmethrin, Tetramethrin, Transfluthrin

10. **Insect growth regulators (IGR)**

i) **Chitin synthesis inhibitors**, e.g., Bistrifluron, Buprofezin, Chlorfluazuron, Teflubenzuron.

ii) **Juvenoids/juvenile hormone mimics**, e.g., Epofenonane, Fenoxycarb, hydroprene, methoprene, Pyriproxyfen.

iii) **Anti-JH/precocenes**, e.g., Precocene I, II, and III.

iv) **Molting hormone agonists**, e.g., Chromafenozide, Halofenozide.

Most fast-acting insecticides act by inhibiting the transmission of nerve impulses and/or activity of neurotransmitters in the nervous system of the insect. The slower acting insecticides inhibit or block specific enzymes in cells or the electron transport chain in mitochondria. Slow-acting insecticides such as insect growth regulators (IGRs) disrupt hormonal action or chitin synthesis in the insect body.

Basically, insecticides have five very broad modes of action

A. Physical poisons—dusts, fumigants, and oils. These poisons kill insects by asphyxiation, that is, blocking the flow of oxygen through the insect tracheal (respiratory) system.

B. Protoplasmic poisons are inorganic chemicals that physically destroy cells.

C. Metabolic inhibitors either interfere with metabolic pathways or inhibit certain enzymes.

D. Neuroactive agents affect the transmission of nerve impulses or the neurotransmitter.

E. Insect growth inhibitors disrupt growth and the development or malformation of cuticle.

The specific modes of action encountered by commonly used insecticides are

1. Block deactivation of acetylcholine esterase in nerve synapse.
2. Action on synaptic receptors.
3. Non-competitive blocking of GABA-gated chloride channels.
4. Blocking of sodium channels in nerve axon.
5. Affecting voltage-dependent sodium channels (sodium channel modulators).
6. Inhibiting the transfer of electrons in the electron transport chain.
7. Uncoupling of oxidative phosphorylation.
8. Inhibition of *adenosine triphosphatase (ATPase).*
9. Juvenile hormone and its mimics (juvenoid-IGR insecticide).
10. Inhibitors of chitin synthesis (chitin inhibitor–IGR insecticides.
11. Inhibition of prothoracicotropic hormone (PTTH) (PTTH inhibitor–IGR Insecticide)

Most commonly used insecticides in India are organophosphate insecticides such as Malathion, Parathion, Chlorpyrifos, Monocrotophos, Diazinon and Cypermethrin. Monocrotophos is one of the most commonly used organophosphate insecticides in Tamilnadu. Monocrotophos affects the nervous system by inhibiting acetylcholinestrase, an enzyme essential for normal nerve impulse transmission. [(10)]

Studies have been reported that long term exposure to insecticides can cause abnormalities such as Reproductive toxicity [(11),] Carcinogenicity [(12)], and Genotoxicity [(13)(14)]

Monocrotophos [dimethyl (E)-1-methyl-2-(methyl carbamoyl) vinyl phosphate, MCP], commonly known as Azodrin and aliphatic organophosphate of class I B [(8)] and highly toxic. It is an important broad spectrum systemic organophosphate pesticide, widely used in agriculture and animal husbandry throughout the world including India.

Organophosphorus insecticides induce production of reactive oxygen species (ROS) and extensive data suggest that oxygen free radical formation can be a major contributor to the toxicity of pesticides. Organophosphorus insecticides may enhance lipid peroxidation by directly interacting with biomembranes. Some investigators have suggested that lipid peroxidation is one of the molecular mechanisms involved in organophosphate insecticides induced toxicity.

Monocrotophos was extremely toxic by oral route for rats and mice, with $LD_{50}$ values of approximately 8 and 10 mg/kg respectively.[15] Monocrotophos is known to cause reproductive toxicity by significantly reducing the sperm count and motility, decreases the reproductive hormone (Testosterone, Luteinizing hormone, follicular stimulating hormone) levels.[11] Its also affect the female reproductive system.[16]

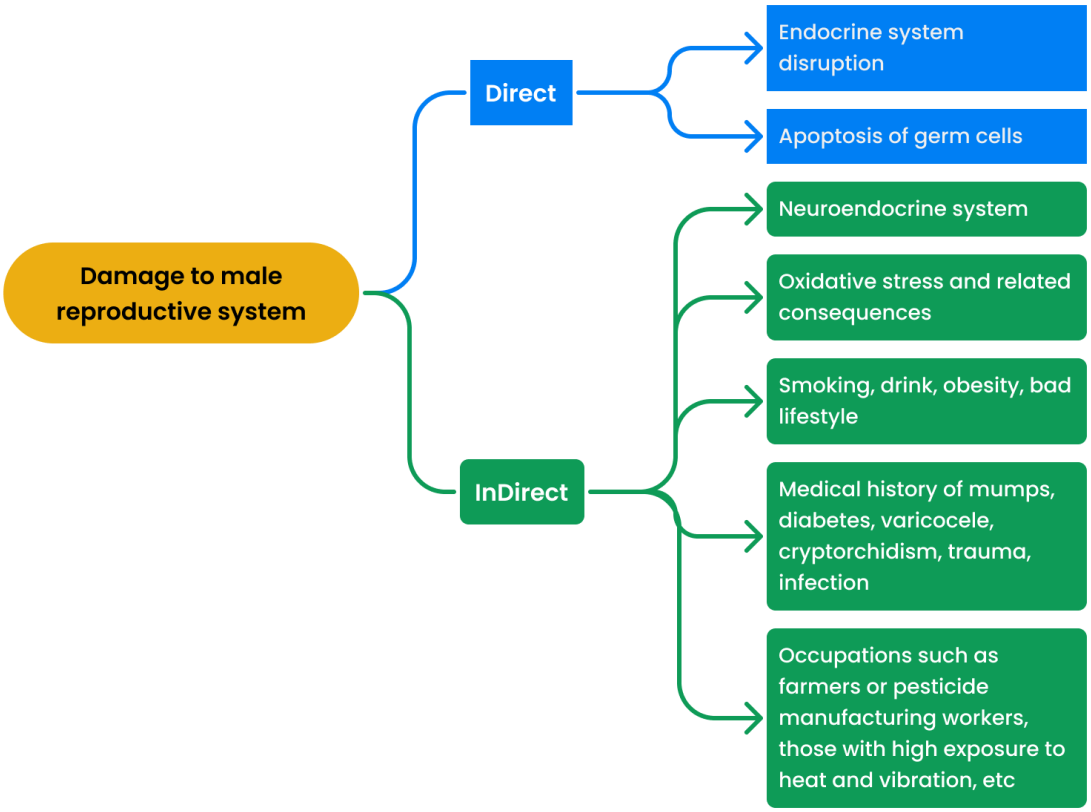



**Figure.1.Direct and indirect deleterious effects on male reproductive system**

# DISEASE PROFILE

# 2. DISEASE PROFILE

## 2.1 MALE INFERTILITY

### 2.1.1 Definition

The World Health Organization (WHO) defines infertility as the inability of a sexually active couple to achieve pregnancy despite unprotected intercourse for a period of greater than 12 months. [2]

Causes of infertility in men can be explained by deficiencies in ejaculate volume causing low sperm production (Oligospermia), poor sperm motility (asthenospermia), abnormal morphology (teratospermia), and abnormal sperm function or by preventing sperm transport to vagina. [17]

## 2.2 CLASSIFICATION

Male infertility is mainly categorized into [18]

- Azoospermia
- Coital infertility

### 2.2.1 Azoospermia (AS)

AS is complete sperm absence in the ejaculate. It is recognized in 15% infertile men and is classified into Obstructive Infertility (OI) and Non-obstructive Infertility (NOI) [19] [20]

- **Obstructive infertility (OI)**

In OI the ejaculate is devoid of spermatozoa with normal spermatogenesis. Approximately 40% of azoospermia cases suffer from OI. It is characterized by normal endocrine and exocrine system along with normal testes spermatogenesis. [21] However, there is an obstruction in the genital tract (GT)). It might also occur in any part between ejaculatory ducts and testes. [22]

- **Non-obstructive infertility (NOI)**

About 60% of azoospermia patients suffer from NOI which is characterized by abnormal spermatogenesis. It results from primary or secondary testicular failure (TF) or partial or vague testicular failure [23]

### 2.2.2 Coital infertility (CI)

It is characterized by normal sperm production and genital tract. Yet, the illness is secondary to patient's sexual dysfunction, that effects ejaculation [18]

## 2.3 CAUSES OF MALE INFERTILITY

Male infertility can be due to multiple reasons including;

Varicocele (enlargement of spermatic veins), testicular failure, endocrine dysfunction, genital tract infection, testicular disturbances, testicular cancer, exposure to gonadotoxic substances [(24)]prolonged exposure to heat, obesity, smoking, older age, hormonal disturbances, retrograde ejaculation (ReE), impotence [(25)], environmental pollutants (i.e. lead, paint, radiations, pesticides) [(26)],wearing tight underwear (increases scrotal temperature results in decreasing the sperm production) [(27)] and vitamin C in diet, excessive stress, malnutrition, anaemia and use of certain drugs including nitrofurantoin, spironolactone and cemitidine [(28)]

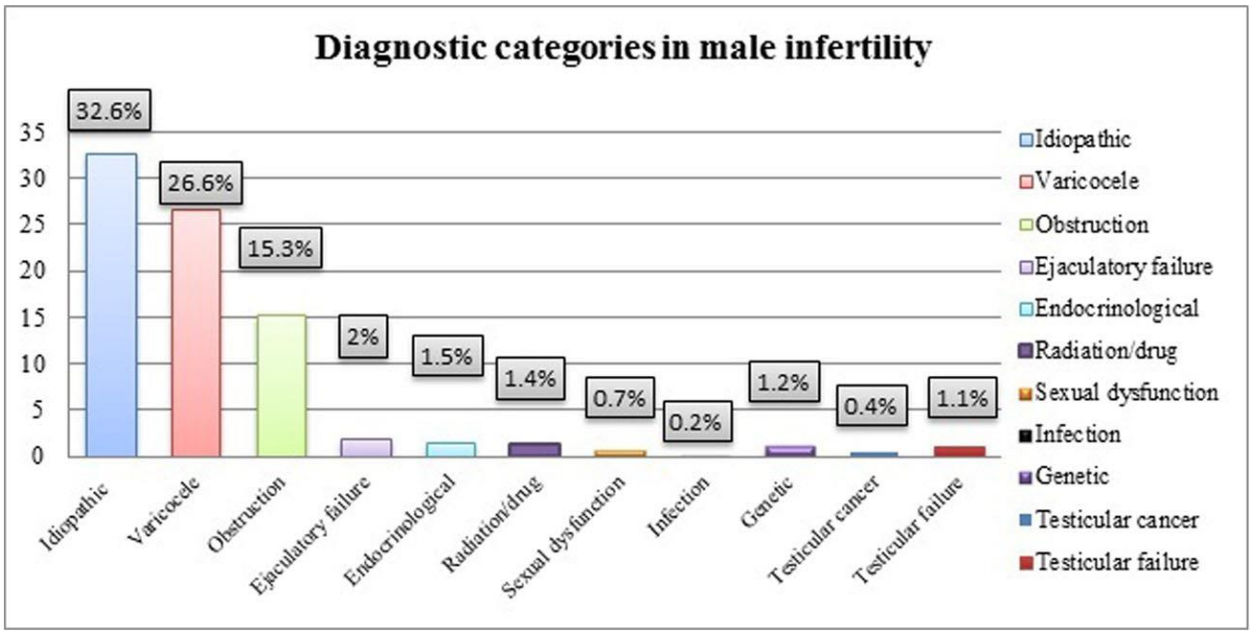



**Figure: 2. Various causes of male infertility**

## 2.4 RISK FACTORS OF MALE INFERTILITY

### 1. Age

Many studies have reported decrease in male fertility with increase in age due to changes in all sperm parameters with the increasing age there is reduction in sperm concentration, sperm motility and seminal volume. [(29)(30)(31)] Erectile dysfunction (EDF) is also common in aged men. The prevalence of EDF is more with increasing age. Approximately 40% men of age 40 suffer from EDF. Occurrence of complete EDF upsurges from 5% to 15% with increasing age from 40 to 70 years [(32) (33)]

**2. Genital tract infections (GTI)**

Different types of viral and bacterial infections can influence male fertility by reducing sperm viability. S. aureus and E. coli found in male semen may result in primary infertility. [34] Ureaplasma urealyticum (UU) is pathogenic and causes GTI leading to male infertility. [35] Herpes simplex virus (HSV) found in semen may cause low sperm count, reduce sperm motility and sperm damage [34]

**3. Sexually transmitted diseases (STDs)**

STD's are caused by bacteria, viruses and parasitic microorganisms. These diseases are; Syphilis, Chlamydia, Gonorrhea, Chancroid and Trichomoniasis. Human papillomavirus, Lymphogranuloma venereum and HSV also cause STD's which may result in poor quality sperm and reduced sperm motility and concentration. [36] However, these effects on semen are unclear. Chronic infections are more likely cause of infertility than acute ones. [37]

**4. Malnutrition**

There is an instinctive relationship between reproduction and nutrition. Nutrition has a vital role in sperm quality [38].Malnutrition might be an imperative cause of male infertility. Prolonged starvation and excessive exercise can affect sperm count, motility and even can stop sperm production. Excessive restriction in food intake can reduce the level of Zinc, vitamin C, vitamin A, vitamin E, selenium (Se), folic acid and other nutrients that are necessary for proper functioning of body and spermatogenesis [39]

**5. Psychological stress**

It is an uncomfortable emotional state accompanying behavioural, biochemical and physiological changes. [40] Stress; whether social, psychological or physical is an attention seeking element of society. [41] Different studies have suggested that psychological stress plays a key role in male infertility. In males, it increases glucocorticoid level which suppresses testosterone concentration in testes that rigorously affecting spermatogenesis. [40]

**6. Drugs**

Impaired reproduction is a multifactorial problem. Some drugs are also involved in causing male infertility. Sulfasalazine is an anti-rheumatic drug, which is known to reduce male fertility. Methotrexate is an immunosuppressive drug that alters semen quality. Methotrexate is responsible for the inhibition of dihydrofolate reductase enzyme which is important for folate synthesis. Deficiency of folate leads to suppression of many cellular processes important for sperm development. Use of beta blockers and some psychotropic drugs can cause impotence in males. Other drugs with high risk of infertility are; cyclophosphamide, chlorambucil, melphalan, procarbazine, bleomycin and dactinomycin [(42)] [(43)]

**7. Chemical agents**

**(A). Heavy metals**

Some metals including lead, chromium, copper and cadmium adversely affect sperm and male reproductive system. Increase level of these metals in the blood affect male fertility, by decreasing the sperm count, reducing sperm quality and altering its morphology. [(44)] [(45)] Welders are more exposed to radiant heat, deleterious metals and harmful gases and have lesser sperm count and viable sperms. [(23)]

**(B). Pesticides**

Rate of male fertility is declining worldwide. Pesticides are another contributing factor in male infertility. Experimental evidences have shown that chemicals present in pesticides can cause testicular cancer, reduce sperm quality and erectile dysfunction. Individuals exposed to pesticides have high risk of primary and secondary infertility. These chemicals may block the activity of hormones i.e. androgens, testosterones, and gonadotropins which influence male reproductive system. [(46)] [(47)]

**8. Excessive heat**

Spermatogenesis is influenced by temperature. Testicles lie outside the body in scrotum, because production of active sperms requires 3–4 °C lower temperature than body temperature. Testicular hyperthermia can cause the genital heat stress leading to the production of low quality spermatozoa. Heat stress leads to damage DNA, autophagy and apoptosis of germ cells due to generation of reactive oxygen species and breakage of strands. [(48)] [(49)]

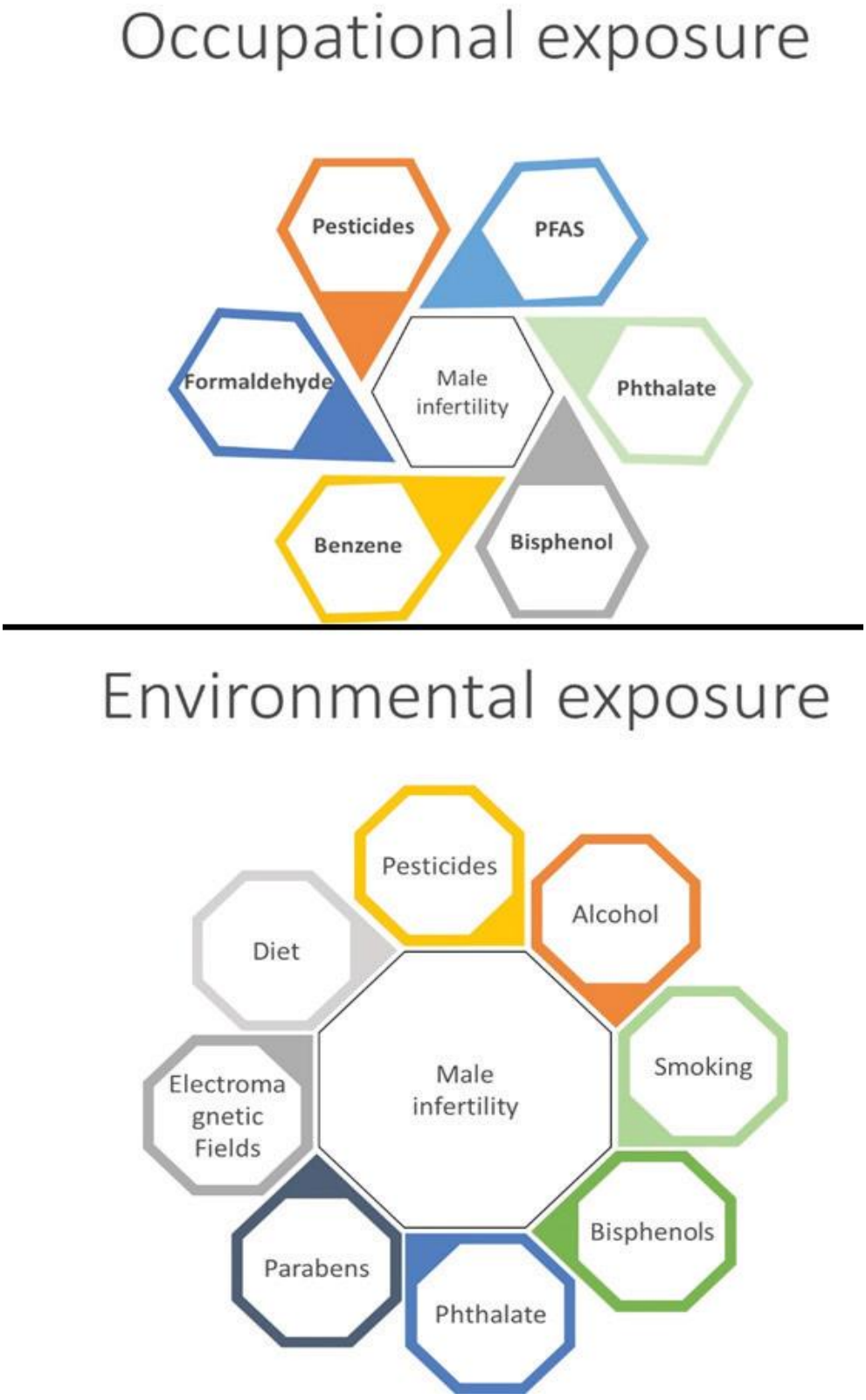



**Figure: 3. Overview of occupational and environmental stressors associated with male infertility**

**9. Posture and clothing**

Testicular temperature is influenced by scrotal position. It changes with the change in posture. Scrotal temperature is low on a naked and upright body Researchers have shown that wearing tight clothing and tight underwear also increases scrotal temperature [27] [49]

**10. Exercise**

Excessive exercise can also elevate body temperature of men's testicles and hence can cause hyperthermia which subsequently produces heat stress of germ cells and sperm damage. [49] Among exercises, frequent bike riding and cycling can also increase testicles temperature, due to body posture, intensity and duration of ride or cycling [27]

**11. Laptop use** [50]

Men who keep their laptops on their legs are also at high risk of infertility problems because of increased scrotal temperature due to heat from pressing of legs together and the heat from laptop

**12. Seasonal temperature**

Seasonal temperature affects sperm concentration, motility and morphology. [49] There are evidences on reduction of sperm count in summer. Researchers have reported that it varies by 70% in winter. [51]

**13. Smoking**

Cigarette smoke is a vital source of non-occupational exposure to metals that produces hazardous effects on male fertility. It comprises of approximately 30 toxic metals, among them lead, cadmium and arsenic are in great concentrations. [52] Cigarette is also a source of nicotine. By-products of nicotine reduce the fertilization capacity of sperm by reducing the motility of sperms. There is high concentration of free radicals in the seminal fluid of smokers. [26]

**14. Alcohol**

Use of alcohol adversely affects male reproductive system in a number of ways. [26] Many evidences have shown that excessive intake of alcohol in men causes shrinkage of testes and impairs production of testosterone hence resulting in infertility, impotence, decreased libido and reduction of secondary sexual characteristics It also affects sexual performance by depressing central nervous system thus causing difficulty in erection and controlling ejaculation. [53]

**15. Obesity**

Obesity adversely affects male fertility by suppressing spermatogenesis and sperm morphological changes. [54] Obesity affect fertility of men by changing hormonal profile, increasing scrotal temperature and altered semen parameters. In obese men, there is increase distribution of fat in upper thighs, scrotum and suprapubic area, which is associated with increase testicular temperature that affects spermatogenesis. [49]

**16. Oxidative stress**

Oxidative stress caused by a high amount of reactive oxygen species (ROS) has been observed in 30–80% of infertile patients. High levels of ROS promote impairment of sperm quality mainly by decreasing motility and increasing the levels of DNA oxidation, protein oxidation and lipid peroxidation. ROS represent a major factor that contributes to male idiopathic infertility. In recent years, male oxidative stress infertility (MOSI) emerged as a term defining "infertile men with abnormal semen characteristics and oxidative stress" that has a global incidence of about 37.2 million cases. MOSI is characterized by abnormal semen parameters with no clear cause of idiopathic infertility. Oxidative stress is an important cause of male infertility due to detrimental changes during spermatogenesis, epididymal maturation, and sperm capacitation that can lead to infertility [55] [56]

**Physiological role of ROS on sperm**

For optimal sperm function and for fertilisation to occur, there must be a balance of ROS and antioxidants. ROS induces cyclic adenosine monophosphate (cAMP) in spermatozoa that inhibits tyrosine phosphatase leading to tyrosine phosphorylation. In particular, $H_2O_2$ stimulates

capacitation via tyrosine phosphorylation triggering a cell signalling cascade. Capacitation not only requires ROS, but it can be inhibited by catalase (CAT). It has been described that high levels of ROS promote the acrosome reaction, whereas the presence of CAT or superoxide dismutase (SOD) inhibits the acrosome reaction. The mechanism of inducing the acrosome reaction appears to be ROS-modulated tyrosine phosphorylation. Motility can also be affected by ROS. Hyperactivation is increased when spermatozoa are exposed to ROS. ROS-mediated tyrosine phosphorylation in the flagellum causes hyperactivation. Tyrosine phosphorylation also augments sperm membrane binding to the zona pellucida ZP-3 protein, promoting sperm–oocyte fusion.

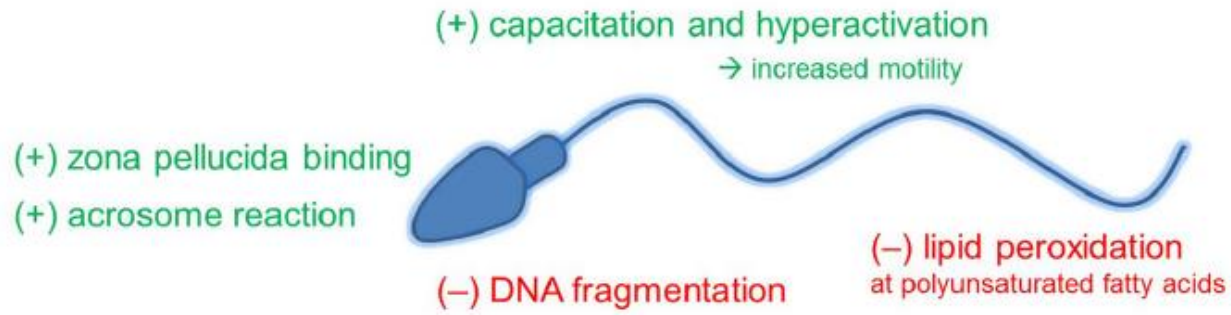



**Figure: 4. The physiological (+) and pathological (-) roles of oxidation in spermatozoa**

**Pathological effects of ROS on spermatogenesis and function**

Although ROS are necessary for normal physiological function of sperm, excessive oxidative stress can cause increased susceptibility of DNA damage, potentially leading not only to infertility, but also recurrent pregnancy loss or genetically heritable mutations causing paediatric diseases. Due to the high susceptibility of unsaturated fatty acid content of sperm to oxidative stress, extensive membrane damage can occur. Once lipid oxidisation has been initiated, the double bonds of an unsaturated fatty acid are attacked by a free radical to create a lipid peroxide radical. The lipid peroxide radical reacts with the neighbouring lipid molecule triggering a chain reaction that can lead to >50% oxidation of the sperm plasma membrane. Eventually lipid peroxidation stops once two lipid radicals react with each other thereby reaching stability.

By-products of lipid oxidisation include mutagenic and genotoxic molecules malondialdehyde (MDA) and 4 hydroxy-nonenal (4-HNE) leading indirectly to DNA damage. Free radicals have the ability to directly damage sperm DNA by attacking the purine and

pyrimidine bases. ROS cause damage via single and double strand DNA breaks, cross links, and chromosomal rearrangements. Most of the sperm genome (85%) is bound to central nucleoprotamines that protect it from free radical attack. Infertile men often have deficient protamination, which may make their sperm DNA more vulnerable to ROS damage. Another mechanism of spermatic DNA damage is free radical-initiated apoptosis, leading to caspase-mediated destruction of DNA. Spermatozoa have a limited ability to repair DNA and can only perform repairs during specific stages of spermatogenesis. Repair mechanisms in haploid spermatozoa are necessary to allow chromatin remodelling. Interactions of sperm with an oocyte may allow for some repairs of DNA, which can affect fertilisation and possible pregnancy. Decreased motility has been shown to be due to ROS-induced, primarily $H_2O_2$-mediated, peroxidation of lipids in the sperm membrane decreasing flexibility and by inhibition of motility mechanisms. The reduction in sperm motility is proportional to the amount of lipid peroxidation. ROS-induced damage of mitochondrial DNA leads to decreased ATP and energy availability, impeding sperm motility. Studies have shown higher levels of ROS in patients with oligospermia. High levels of ROS disrupt mitochondrial membranes, leading to activation of caspases and ultimately apoptosis. Cytochrome C release during the apoptotic pathway further increases levels of ROS, promoting DNA damage and fragmentation, and potentially augmenting the apoptotic cycle. Sperm with higher levels of oxidative stress, especially after freeze/thaw cycles, have higher levels of caspase activation that can trigger apoptosis. [55][56]

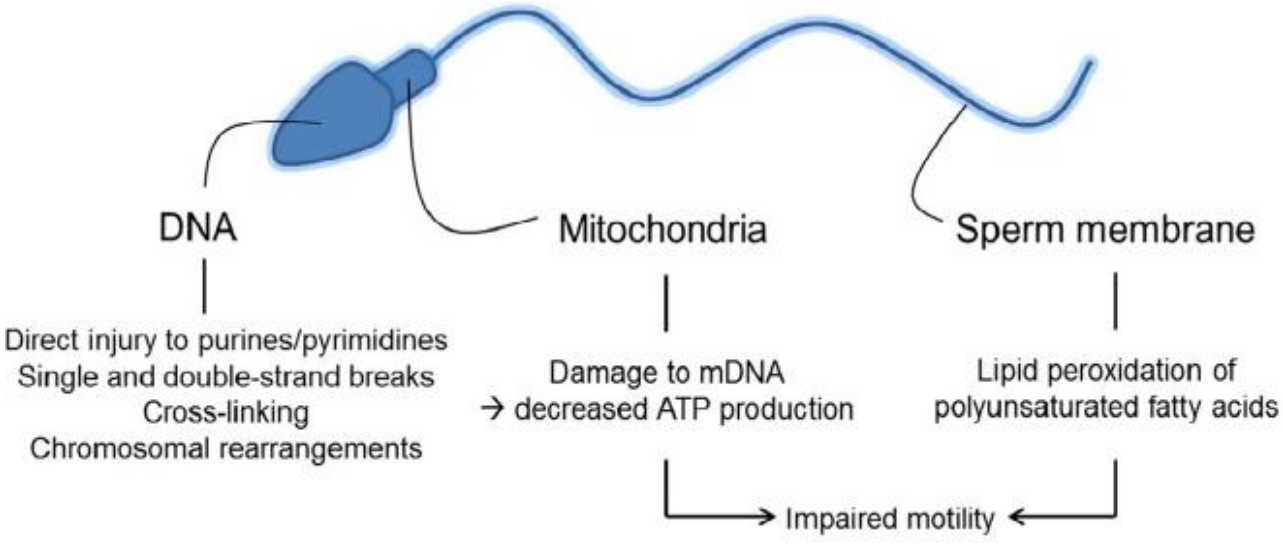



**Figure: 5. Oxidative stress and injury to DNA, mitochondria and plasma membrane of spermatozoa.**

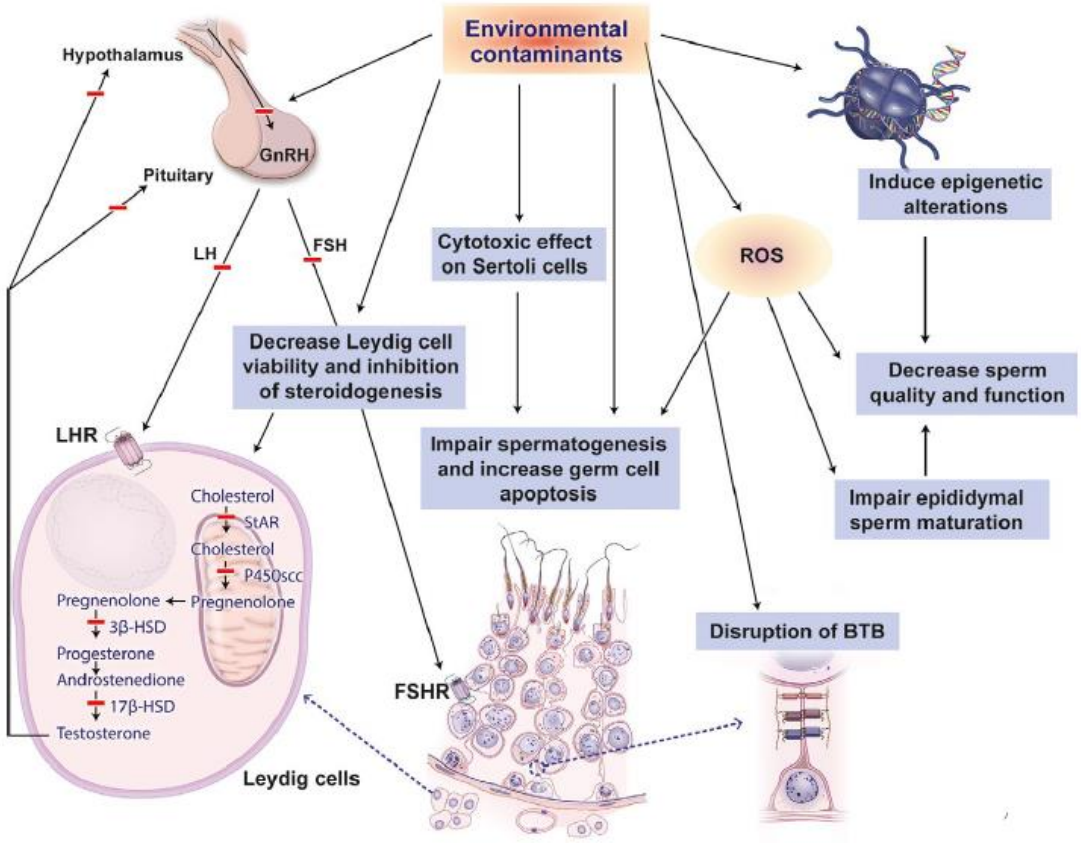



**Figure: 6. Mechanism of action of environmental contaminants causing male reproductive toxicity**

## 2.5 Diagnostic tools for male infertility

### 1. Physical examination

Case history and physical examination are important tools for the disease assessment. It involves patient questioning, genital examination including prostate, anal sphincter tone and bulbocavernosus reflex evaluation. [57] Physical examination involves assessment of cryptorchidism (UCr/BCr), genital tract infection, testicular cancer, testicular torsion, testicular trauma, absence of testes, gynaecomastia, varicocele, abnormal sexual characteristics and abnormal testicular volume or consistency [58]

**2. Semen analysis**

It is a main tool for analysis of male infertility. It should essentially be carried out at high standards to estimate all parameters of male ejaculate. Computer assisted semen analysis (CASA) is considered mainly a research tool and is not used routinely. If abnormal results are obtained semen analysis is repeated after 6-12 weeks. [57] [59] [60]

**Table no. 1. Parameters of semen analysis**

| | |
|---|---|
| **Semen volume** | Normal ejaculate volume is 1.5- 5 ml. |
| **Colour** | Grey, yellow or opalescent |
| **pH** | 7.2 – 8 |
| **Liquefaction** | Coagulation occurs soon after ejaculation but semen liquefies within 5-20 minutes failure to liquefy after 30 min is abnormal |
| **Sperm concentration** | Sperm per ml of semen<br>Normal - 20 x $10^6$ sperms / ml<br>Oligozoospermia - 20 x $10^6$/ ml<br>Polyzoospermia - 350 x $10^6$/ min |
| **Total sperm count** | Sperm concentration x volume of semen<br>Normal value - 40 x $10^6$ sperms /ejaculate. |
| **Sperm Motility** | At least 100 sperms are evaluated. Normal is 50 % with forward progression within 60 min of ejaculation |
| **Morphology** | Assessed by light microscopy (hematoxylin, eosin, geimsa or papanicoloau stain) or electron microscopy. At least 100 sperms are examined. Normal value - 30 % with normal forms |
| **White blood cells** | Have to be differentiated from immature germ cells.<br>Normal (1 x $10^6$ / ml peroxidase staining technique).<br>If excessive, semen culture should be performed |
| **Sperm antibodies** | Detected by immunobead or mixed antiglobulin reaction<br>which localizes IgG or IgA specific regions of spermatozoa.<br>Normal for immunobead test for antiglobulin reaction test –<br>10% spermatozoa with adherent particle. |

**Collection of semen**

- The sample should be obtained atleast in three occasions.
- With an interval of atleast two months of each specimen.
- Atleast 4 days abstinence from sexual activity.
- A sample to be collected by masturbation into a clean, dry, sterile container.
- The specimen (semen) should be examined for above mentioned parameters.

**3. Hormone tests**

Hormonal imbalance can also cause male infertility. It can be checked from blood sample [62] The test is limited in determining the levels of testosterone, LH and FSH. It is performed in individuals with possibility of hypogonadism. The differentiation between obstructive infertility and non-obstructive infertility is a significant factor in male infertility. In obstruction there is normal level of FSH with bilaterally standard testicular volume. Yet, 29% individuals having normal FSH are characterized by defective spermatogenesis or spermatogenic arrest. [57]

**4. Testicular biopsy (TBO)**

TBO is the only technique for testicular histopathology. The biopsy is performed under anesthesia. Bilateral TBO is recommended while diagnosing male infertility. It is predominantly useful for investigation of oligospermia and AS with normal endocrine activity. TBO for oligospermia and AS represents altered pathological patterns. Among different patterns, hypospermatogenesis is the common spermatogenic defect pattern. Though sertoli cell only (SCO) histology is a common pattern in individuals with AS, small testes, primary infertility and primary testicular failure. High LH and FSH and Low testicular volume are associated with compromised spermatogenesis. [63]

Men with $\geq$ 7.6 mIU/ml FSH or $\leq$ 4.6 cm testicular long axis suffer from NOI. However, Men with $\leq$ 7.6 mIU/ml FSH or $\geq$ 4.6 cm testicular long axis may undergo reconstructive surgery either with or without TBO and sperm extraction, or TBO only [64]

**5. Urine test**

This is a non-invasive and cost effective technique for screening males with reproductive impairment. The test is for total FSH in urine and is decidedly sensitive to identify males with high serum FSH. Men with low urine FSH, also represent low serum FSH. Serum FSH increases in testicular failure which causes azoospermia. Post ejaculation urine assessment can also identify men with retrograde ejaculation. [63]

## 2.6 TREATMENT FOR MALE INFERTILITY

Some of the options for treating infertility are surgery, treatment of infections, treatment of second intercourse problem and hormone treatment. Alternative medicines are also popular in the treatment of male infertility. Though evidence is limited, they are used by a large community of men. Herbal drugs are popularly used to treat male infertility.

**1. Surgical treatment [65]**

- Obstructive Azoospermia
- Surgical sperm recovery (SSR)
- Intrauterine insemination (IUI)/Donor insemination (DI)
- IVF/ICSI
- Intracytoplasmic morphologically selected sperm injection (ICMI)
- Physiological Intracytoplasmic sperm injection (PICSI)

**2. Hormonal treatment [66] [67] [68]**

Hormonal therapies, such as human menopausal gonadotrophin (HMG)/human chorionic gonadotrophin (HCG), androgen, anti-oestrogens (clomiphene and tamoxifen), prolactin inhibitors (bromocriptine) and steroids

- **Gonadotropin-releasing hormone (GnRH) [67] [68] [69]**
- **Gonadotropins [67] [68]**
- **Dopamine agonist [67] [69]**
- **Aromatase inhibitor (AI) therapy**
- **Selective estrogen receptor modulators (SERMs) [67]**

**3. Antioxidant [71]**

Vitamins E, C, A, B complex, coenzyme Q10 (CoQ10), ubiquinol, glutathione, L-carnitine, lactoferrin, β-carotene, lycopene, pantothenic acid, α-lipoic acid, N-acetyl-cysteine, selenium, zinc, copper or supplements containing a combination of these antioxidants [70].

Carni-Q-Nol (440mg L-carnitine fumarate + 30mg ubiquinol+ 75 IU vitamin E + 12 mg vitamin C) softules twice or thrice daily decreased sperm pathology after 3 months, improved sperm density after 3 and 6 months, increased (ubiquinone + ubiquinol) & α-tocopherol and decreased oxidative stress levels. Apart from improving sperm parameters, the supplementary therapy with Carni-Q-Nol resulted in a 45% pregnancy rate.[71]

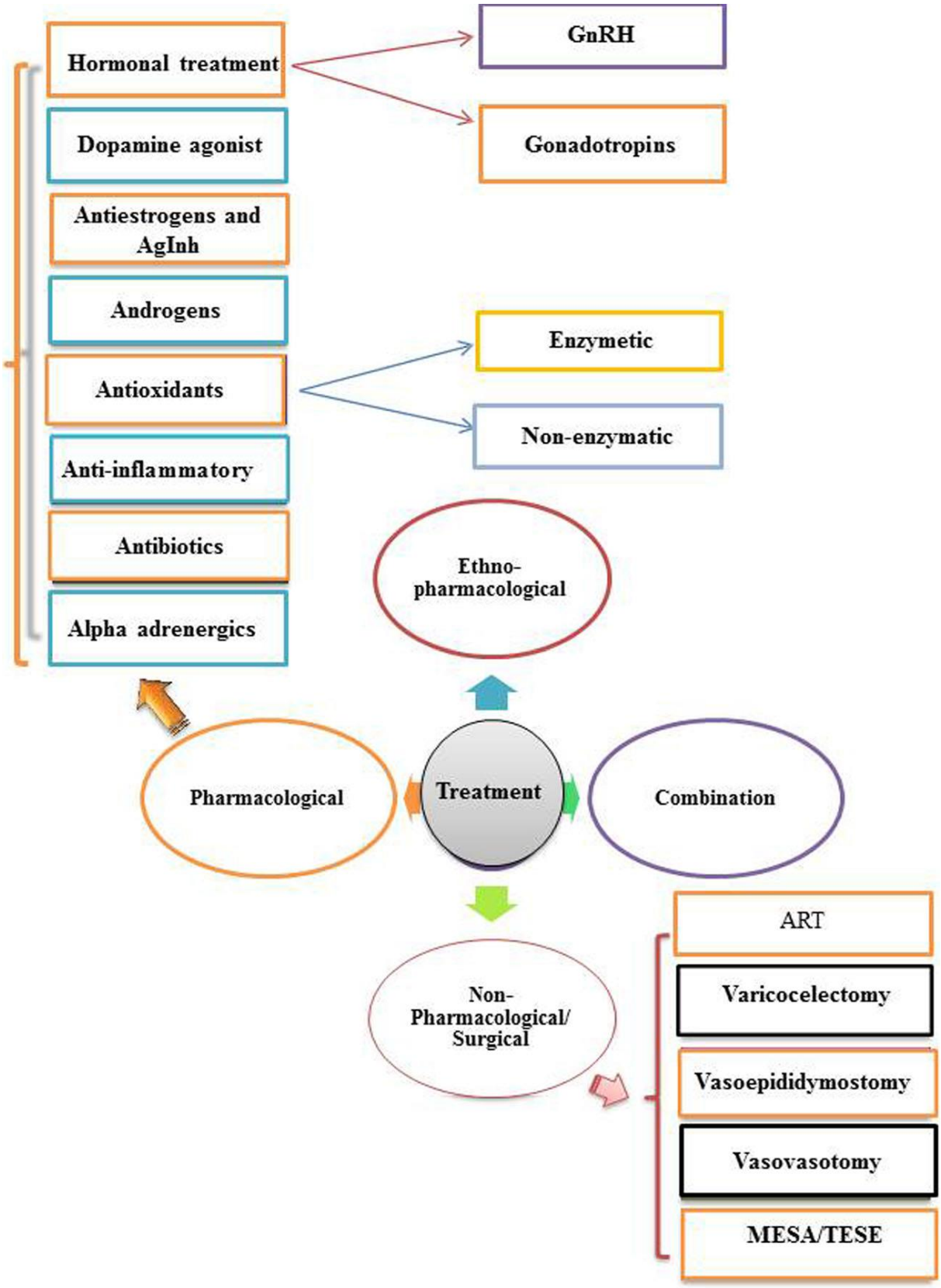



**Figure .7. Treatment for male infertility**


# LITERATURE REVIEW

# 3. LITERATURE REVIEW

## NAGA PARPAM

**Balakrishnan, Ilango *et al* (2009)** [72] In this study, A zinc-based preparation of Siddha medicine, *Naga Parpam* (*NP*), is prescribed in the treatment of a variety of diseases. In folklore practice, *NP* is added as an ingredient in medicaments to treat obesity. Hence it was studied for anti-hyperlipidemic activity in cholesterol fed rats with continuous administration for 30 days in doses of 10 and 20 mg/kg body weight. The drug prevented the elevation of total cholesterol, triglycerides, low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) in serum, liver and heart tissues in a dose-dependent manner when compared to control animals.

**Arun Sudha *et al* (2009)** [73] In this study , the characterization of some of the metal based herbal medicine, *Naga Parpam* which is used to treat piles, fistula, diarrhoea, cough, asthma and dysentery. It is administered along with butter or ghee or Thetran kottai lehyam in the dose of 100-200 mg twice a day. ZnO nanoparticles are proved to be effective against both Gram-positive and Gram-negative bacteria. They have antibacterial activity against even high-temperature resistant and high-pressure resistant spores. There appears to be an interaction between the bacterial cells and the ZnO particles, which is presumably due to the electrostatic forces

**Willi Paul *et al* (2012)** [74] In this study, the L929 fibroblast cells were seeded in 24 well plates at a density of 5 x $10^5$ cells/well, cultured for 24 h in incubator at 37° C under 5% $CO_2$. The medium was replaced with *Naga Parpam* particle suspension in the medium at a concentration of 5 mg/ml/ well and incubated for 20 hrs. Medium alone was used as control. The particles were removed and 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was done. The results revealed that *Naga Parpam* is slightly toxic. As compared to control (medium) *Naga Parpam* exhibited 75 ± 5% cell viability

**Viswanathan *et al* (2014)**[75] In this study *Naga parpam*, a herbal preparation enriched with Zinc oxide, is reported to be effective in treating pyelonephritis at 600 mg/kg body weight. However, the continuous usage of this drug at high concentrations could lead to nephro-toxicity. Therefore

more studies on optimising the dosage of this drug are warranted on the safe usage of this dug for longer periods of time.

**R. Sahulhameed *et al* (2015)** [76] The aim of this study was to standardize the physico-chemical traits of the *Naga Parpam*, a Siddha traditional drug for treating oligospermia so as to attain maximum benefit to the mankind. The organoleptic characters, physico-chemical characters like ash values, pH value, specific gravity, solubility were analyzed. The total ash value was found to be 27.2%w/w, acid insoluble ash value is 11.6%w/w and loss of drying at 105 º c is 0.29 % w/w. The pH value is 9.6. EDAX revealed the content of Zinc, Carbon, Oxygen, Potassium, Iron, Chloride, Potassium and Calcium. The XRF of *Naga Parpam* revealed the elements like zinc, potassium, sulphur in oxide form as well as in elemental form. This study highlights the suitable application of modern standardizing techniques for bringing the herbal formulation into focus.

**Ramanathan R *et al* (2019)** [77] *Naga Parpam* is a Zinc Oxide based nano medicine, used in Siddha system. Physiochemical analysis is done for standardization (i.e FTIR, SEM, EDAX, XRD and PL) studies were done. The morphology shows that in *Naga Parpam* there are different sized particles, which falls in the nano size. Presence of Zinc, Oxygen, Silicon, Magnesium and Potassium has been established by EDAX studies. It is concluded that *Naga Parpam* is a potential candidate for substitution of Zn, in Zn depleted auto immune diseases and also in cancer.

**K. Rajamaheswari *et al* (2019)** [78] The study confirms that the novel siddha formulation *Nagarasa parpam* has promising effect of anti-cancer activity. SEM images confirmed that the particles of the trial drug *Nagarasa parpam* are found in nano and near nano range. It is concluded that the nano formulated siddha drug *Nagarasa parpam* exhibits potent anti-cancer effect on hep2 cell lines. This research work found as a base for the use of siddha drug *Nagarasa parpam* to treat cancer.

**ZINC**

**Hai-Tao *et al* (2020)** [79] This study aimed to explore the effect of zinc supplement on the reproductive toxicity caused by sub-chronic MIXPs exposure (160 mg/kg/day, for 90 days) in male rats. Testosterone (T), FSH and LH in serum, early toxicity indicators in urine, PIWI proteins (PIWIL1 and PIWIL2) expression in testes and pathological examination were

performed for toxicity evaluation. The results indicated that zinc supplement could inhibit the T, LH, FSH level decreases in serum, abolish the effect of 5 early toxicity indicators' levels in urine, restrain the alteration of PIWI proteins expression and improve the constructional injury of testes.

**Azam Javadi *et al* (2020)**[80] In this study the Extensive application of zinc oxide (ZnO) nanoparticles (NPs) results in increased exposure to these NPs guarantee sperm production throughout the male reproductive life. Short time (one day) exposure of spermatogonial stem cells (SSCs) to a low concentration of ZnO NPs (10 μg/mL) promoted expressions of specific genes (*Plzf*, *Gfr α1* and *Bcl6b*) for SSC self-renewal and differentiation genes (*Vasa*, *Dazl*, *C-kit* and *Sycp3*).

**Viswanathan Vinothaa *et al* (2019)**[81] In this study the synthesis of *Ci*-ZnO NPs (*C. igneus*-coated zinc oxide nanoparticles) were specified using UV–Visible, FTIR, XRD, and TEM. The α-amylase and α-glucosidase inhibition assays demonstrated antidiabetic activity of *Ci*-ZnO NPs, and the DPPH [2,2-diphenyl-1-picrylhydrazyl hydrate] assay demonstrated the antioxidant activity of the nanoparticles (75%). The *Ci*-ZnO NPs exhibited promising antibacterial and biofilm inhibition activity against the pathogenic bacteria *Streptococcusmutans* etc

**Siba Soren *et al* (2018)** [82] In this study the synthesis, characterization, and evaluation of antimicrobial and antioxidant potential of monodispersed Zinc Oxide (ZnO) nanoparticles synthesized by aqueous phase and polyol method. Both the methods had shown excellent DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity (SAS) in the range of 25–75 ng $ml^{-1}$. The antibacterial activities against two Gram-positive bacteria Streptococcus mutans (MTCC 497) and S. pyogens (MTCC 1926) were studied.

**S. Rajeshkumara *et al* (2018)**[83] This study proposes to biosynthesize zinc oxide nano particles (ZnO NPs) from *Mangifera indica* (mango) leaves which were then evaluated for their antioxidant activity on lung cancer A549 cells. The antioxidant potential of nanoparticles was estimated using a DPPH free radical scavenging assay. The MTT assay used for cytotoxicity

evaluation depicted the significant cytotoxic effect of ZnO NPs against the A549 lung cancer cell line. Antioxidant activity of NPs was increased by increasing the concentration of NPs.

**Puran Badkoobeh *et al* (2013)**[84] In this study 24 adult male Wistar rats were divided into four groups (6 rats per group). They received saline (as control), DOX alone (6 mg/kg body weight, i.p.), nZnO alone (5 mg/kg body weight, i.p.), and nZnO followed by DOX. Animals were sacrificed 28 days after treatment and evaluations were made by sperm count and measuring sex hormone levels in plasma. Also total antioxidant power (TAP) and lipid peroxidation (LPO) in plasma were tested. Data was analyzed with SPSS-14 and one way ANOVA test. $P<0.05$ were considered to be statistically significant.

**MONOCROTOPHOS**

**Vismaya *et al* (2014)**[85] In this study the potential of Monocrotophos (MCP) to induce intestinal dysfunction in rats was investigated. MCP was administered orally to rats at sublethal doses (0.45, 0.9 and 1.8 mg/kg b.w/d) for 30 days. MCP increased the activities of intestinal disaccharidases, alkaline phosphatase, glycyl-glycine dipeptidase, and $Na^+/K^+$-ATPase while it decreased cholesterol: phospholipid ratio. They provide evidence that repeated oral intake of MCP has the propensity to alter small intestinal structure and functions, which might lead to intestinal dysfunction.

**Vibhuti Mishra *et al* (2014)**[86] The aim of this study to evaluate the genotoxic potential of Monocrotophos & Quinalphos using micronucleus assay in rats. Both acute and chronic exposure caused DNA damage in rat tissues and lymphocytes as evident from the statistically significant increase in DNA damage index on pesticide exposure. The co-treatment of melatonin has the ability to reduce the genotoxic potential of MCP and QNP under the experimental conditions applied in the present study.

**Xiaona *et al* (2013)**[87] Zebrafish (Danio rerio) were exposed to 0.001, 0.010, and 0.100 mg/L of 40% Monocrotophos pesticide for 40 days post-hatching. Histological analyses were performed to determine whether sex differentiation in zebrafish was affected by Monocrotophos. The results revealed a prominent increase in the proportion of females (71%) in the 0.100 mg/L MCP pesticide treatment as well as the presence of one intersex individual in each of the groups

exposed to 0.001 and 0.100 mg/L MCP. In addition, MCP treatment increased the transcription of brain aromatase (cyp19a1b), resulting in an indirect impact on sexual differentiation

**G. Velmurugan *et al* (2013)**[88] The present study investigated the cardiotoxicity of prolonged intake of Monocrotophos (MCP). Wistar rats were administered 1/50th of $LD_{50}$ dosage of MCP (0.36 mg/kg body weight) orally via gavage daily for three weeks. The cardiac markers (cTn-I, CK-MB and LDH) showed an elevated expression in blood plasma, which signals the cardiac tissue damage. Thus the study shows the cardiotoxic effect of prolonged MCP intake in rats and suggests that MCP can be a possible independent and potent environmental cardiovascular risk factor.

**S.C. Joshia, and B. Bansala (2012)**[89] Male albino rats (Wistar strain) were orally administered with daily dose (1.5mg/kg -1) of Monocrotophos for 30 and 45 days. Sex organ weight analysis, fertility, biochemical, enzymatical, hormonal and histopathological parameters were the criteria used to evaluate the toxicity of the Monocrotophos. Study shows significant decline in the weight of testes, epididymis, seminal vesicle, ventral prostate and reduction in sperm counts. Histoarchitecture of testes showed changes in seminiferous tubules, inhibited spermatogenesis, degenerative Leydig cells and completely damaged Sertoli cells.

**Amajad Iqbal Kazi *et al* (2012)**[90] Wistar rats were administered Monocrotophos (0.8 LD50) by oral gavage to elicit severe effects of acute poisoning and were sacrificed 2.5 h, 24 h, 7 days, 14 days and 1 month after poisoning. Acetylcholinesterse activity, mRNA and protein were assessed in cortex, striatum, hippocampus and cerebellum. *De novo* synthesis of acetylcholinesterase is associated with increased catalytic efficiency that may contribute in restoring cholinergic function.

**Apurva Kumar R *et al* (2012)**[91] Oral administration of Monocrotophos (1.8 mg/kg b.w., 1/10 $LD_{50}$) caused reversible hyperglycemia in rats with peak increase within 2h after the administration. At 4 h following administration, there was normalization of hyperglycemia and hypercorticosteronemia, marginal attenuation of liver TAT activity and marked increase in liver glycogen content, without spontaneous reactivation of AChE activity in the organs studied.

The results clearly demonstrate the involvement of AChE inhibition in hyperglycemia and stressogenic effects of Monocrotophos in rats following acute exposure.

**Santhosh *et al* (2012)**[92] evaluated the genotoxic potential of Monocrotophos and time-dependent repair of the damaged DNA in rats, using single cell gel electrophoresis or Comet assay. The involvement of oxidative stress was also examined by estimation of thiobarbituric acid reactive substances (TBARS) in the tissues of Monocrotophos exposed rats. The rats were given oral exposure of 4.5 and 9 mg Monocrotophos/kg body weight once as well as 0.3 and 0.6 Monocrotophos mg/kg body weight daily for 60 days. The level of DNA damage was estimated by scoring 50 cells per animal, dividing into five types, types 0, I, II, III and IV. The results clearly indicated that exposure to MCP, acutely or chronically, caused a dose-dependent increase in the number of damaged nuclei.

**B. Kalyan Chakravarthi *et al* (2009)**[93] The genotoxic and cytotoxic effects of Monocrotophos, an organophosphate insecticide, was investigated on human lymphocytes cultured *in vitro*. Utilizing the trypan blue dye exclusion technique assay, the $IC_{50}$ of monocrotophos was found to be 16 M. Based on $IC_{50}$ value, Monocrotophos was found to be highly toxic to lymphocyte culture. Chromosomal aberrations induced by monocrotophos were determined using karyotyping.

**Radhika P *et al* (2002)** [94] Monocrotophos a organophosphate pesticide was administered orally at doses of 1.6, 3.3, 6.6, 10 and 13 mg/kg body weight/day to normal virgin Swiss albino mice for 30 days. The vaginal smear and body weight of the mice were recorded. The ovaries from each animal was serially sectioned and stained for follicular studies. Interruption in estrous cycle, decrease in healthy follicles and increase in atretic follicles may be due to hormonal imbalance or toxic effects of monocrotophos, which adversely affects reproductive function.

From the literature review, it is clear that Monocrotophos has the ability to induce male infertility and no scientific work has so far been reported on the ability of Naga parpam to reverse this infertility.

## 3.1. DRUG PROFILE

***Naga parpam***


**Figure.8.** ***Naga parpam***

**Synthesis of *Naga Parpam* [95]**

*Naga parpam* which is a Siddha medicine, prepared from zinc. Pure Zn sheets are heated to red hot, kept in a vessel and the juice of *Eclipta prostrata*, is added to it, just as the process of quenching. This is done again and again till Zn becomes a powder. Now this powder is grinded with *Aloe vera* juice and small flakes are covered in clay which is calcinated at low temperature. The temperature is not mentioned. Again, these flakes are taken and grinded with *Aloe vera* juice and flakes are made and again heated as above. This process is done three to four times till the parpam is formed. In the present work, Naga Parpam was procured from a reputed Siddha manufacturer, Indian Medical Practitioner's Co-Operative Society (IMPCOPS) for this study.

**Ingredients**

- Purified Zinc
- Aloe vera
- *Eclipta prostrate*

**Presence of elements in *Naga parpam* [77]**

**Table no.2. Elemental constituents of *Naga parpam***

| Elements | Naga Parpam | |
|---|---|---|
| | Weight % | Atomic % |
| Zn | 67.4 | 34.74 |
| O | 28.86 | 60.78 |
| Si | 2.65 | 3.18 |
| Mg | 0.67 | 0.93 |
| K | 0.42 | 0.36 |
| TOTAL | 100 | 99.99 |

**Medicinal uses [96]**

- *Naga Parpam* is taken with the oil of *Madhuca longifolia* (*Ilupai nei*) and hot water for intermittent fever, nausea, loss of appetite, abdominal pain, diarrhoea, tumour below the knee joint, septic ulcers and severe blood discharge with itching.
- The zinc powder should be used for two months to obtain good result. It is also used for fissures of the tongue.
- It has a specific control over epilepsy, cholera and other spasmodic diseases as whooping cough, asthma, hysteria, dipsomania etc.
- It is a good remedy to check profuse sweating
- For its astringent property it is given in bronchorrhea and in colliquative sweats of phthisis.
- *Naga Parpam* prepared by castor leaf juice (*Ricinus communis*) is useful in the treatment of gastric ulcer and hemorrhoids. It improves spermatogenesis.
- *Naga Parpam* is also used for the treatment of *kapha*, gonorrhoea (*Vettai*), leucorrhoea (*Vellai*) abdominal tumour and hepatomegaly.
- *Naga Chendooram* when given in cow's butter twice a day for 21 days, cures hemorrhoids, anorectal diseases, venereal diseases and bilious disorders.
- In modern medicine also zinc is considered as an essential trace element and an essential component of a large number of enzymes. It has many therapeutic applications in the treatment of various external conditions of the body as a precipitating germicide

# AIM AND OBJECTIVE

# 4. AIM AND OBJECTIVE

The aim of this study is to evaluate the protective effect of herbo-mineral formulation *Naga parpam* against Monocrotophos induced fertility changes in male rats.

- To procure *Naga parpam* from siddha manufacturer, Indian Medical Practitioner's Co-Operative Society (IMPCOPS) for this study
- To perform the primary phytochemical analysis of *Naga parpam*
- To evaluate *in vitro* anti-oxidant activity of *Naga parpam*
- To evaluate the protective role of *Naga parpam* against Monocrotophos induced fertility changes in male rats

The main objective of the study is to investigate whether the herbo-mineral formulation *Naga parpam* improves

- The sperm count
- Sperm motility
- Sperm viability in rats exposed to Monocrotophos.

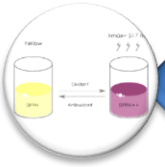

# SCOPE AND PLAN OF WORK

## 5. SCOPE AND PLAN OF WORK

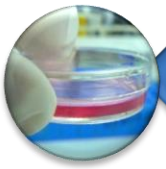
**PROCUREMENT OF *NAGA PARPAM***

**PRIMARY PHYTOCHEMICAL ANALYSIS**

**CHARACTERIZATION OF *NAGA PARPAM***

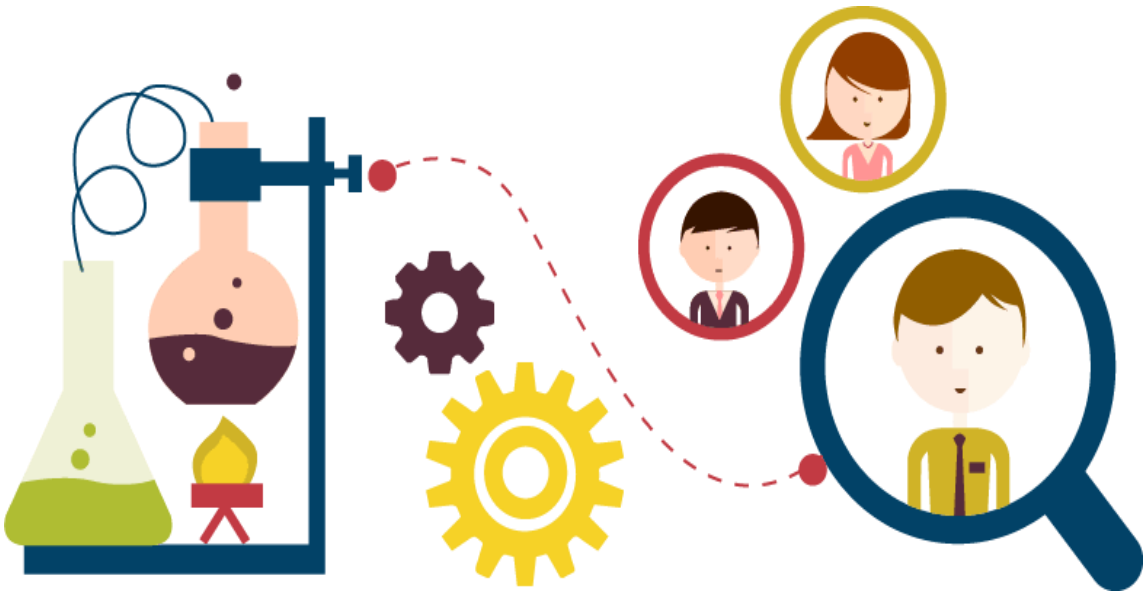


**IN VITRO - ANTIOXIDANT ASSAY**



**IN VIVO PHARMACOLOGICAL STUDIES**

**RESULTS AND DISCUSSION**



# MATERIALS AND METHODS

# 6. MATERIALS AND METHODS

## 6.1 Procurement of Naga parpam

Naga Parpam was purchased from the reputed siddha manufacturer Indian Medical Practitioner's Co-Operative Society (IMPCOPS) in the month of November, 2021.

## 6.2 Organoleptic characters [97]

- **Colour (Varna)**

Every parpam has a specific colour. If the colour of the incinerated material complies with the classical textual reference, then it can be considered as a proper parpam

- **Tastelessness (Niswadu)**

A pinch of parpam has to be placed on the tongue to check its taste since metals in their natural form have a specific taste.

- **Lustreless (Nishchandrata)**

Presence of lustre indicates that the process of parpam preparation was not completed. So as to check this, pinch of parpam was taken and observed under bright sunlight.

- **Lightness (Varitara)**

After proper incineration, the parpam particles become too light and they cannot break the surface tension of the stagnant water. So, they float. Clean water was taken in a glass and allowed to standstill. A pinch of parpam was sprinkled on the surface of water

- **Fineness (Rekhapurnata)**

A pinch of parpam was rubbed in between thumb and index finger. If parpam particle entered into the creases of these fingers; it indicates that the metal is incinerated properly. Finer the particle, fastest the absorption and quickest the action; is the fundamental principle of pharmacology.

- **Smokeless (Nirdhuma)**

A pinch of *Naga parpam* was sprinkled on the ignited charcoal and observed for the fumes

**6.3 Physio-chemical analysis of test drug**

**Quantitative analysis [98]**

**Total Ash**

Incinerate about 2 to 3 g, accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 6000 until free from carbon, cool in a desiccator for 30 min and weigh without delay. If carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 6000. Calculate the percentage of ash with reference to the air-dried drug.

**Calculation:**

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

**Acid insoluble ash**

To the ash add 1:5 (HCl: Distilled water) 15 ml boil, cooled and then filtered using Whatman filter paper (No.41) repeat 3 to 4 times till the yellow colour disappear or colourless, then remove the filter paper and add to the filter to the original dish and keep it in the muffle furnace at 600 $^{o}$ C and take constant weight and calculate the acid insoluble ash value.

Acid insoluble residue = Acid insoluble ash value – Empty weight of the dish

$$\text{Acid insoluble ash (\%)} = \frac{\text{Weight of acid insoluble residue}}{\text{Weight of the sample}} \times 100$$

**Loss on drying**

3gm of the drug is heated in a hot oven at 105 $^{o}$ C to constant weight. The % of weight was calculated.

**Alcohol-soluble Extractive**

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of *alcohol* of specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowing to stand for 18 hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105 º C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

**Calculation:**

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

**Water-soluble Extractive**

5 g of the test drug was weighed accurately in a glass stoppered flask. 100 ml of distilled water and shacked occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in a hot air oven at 105º C for 6 hours. Desiccator was cooled and weighed. The experiment was repeated twice and the average value was noted. The percentage of water soluble extractive was calculated by the formula given below.

**Calculation:**

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

**Potential of hydrogen (pH)**

The pH scale is logarithmic and runs from 0.0 to 14.0 with 7.0 being neutral. Readings less than 7.0 indicate acidic solutions, while higher readings indicate alkaline or base solutions.

**6.4 Phytochemical analysis**

**Methodology for chemical analysis**

**Preparation of extract**

5 g of sample is weighed accurately and placed in a 250 ml clean beaker and added with 50 ml of distilled water. Then it is boiled well for about 20 minutes. Then it is cooled and filtered in a 1000 ml volumetric flask and made up to 100ml distilled water.

**1. Test for Alkaloids**

**a) Mayer's test:** Alkaloids gives cream colour precipitate with Mayer's reagent [Potassium mercuric iodide solution].

**b) Dragendroff's test:** Alkaloids gives reddish brown precipitate with Dragendroff"s reagent [Potassium bismuth iodide solution].

**c) Wagner's test:** Alkaloids gives a reddish brown precipitate with Wagner's reagent [Solution of iodine in potassium iodide].

**d) Hager's test:** Alkaloids gives yellow colour precipitate with Hager's reagent [Saturated solution of Picric acid].

**2. Test for carbohydrate**

**a) Molisch's test:** To the 0.5 ml of extract, added few drops of alcoholic alpha napthol and 0.2 ml of concentrated sulphuric acid slowly through the sides of the test tube, a purple to violet colour ring appears at the junction.

**b) Benedict's test:** The extract was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled on water bath, reddish brown precipitate forms if reducing sugars are present.

**3. Test for Glycosides**

The extract was hydrolyzed with mineral acid and then tested for the glycone and aglycone moieties.

**a) Legal's test:** The extract was treated with pyridine and alkaline sodium nitroprusside solution was added, blood red colour appears.

**4. Test for Cardiac Glycosides**

**a) Keller killiani test [Test for Deoxy sugars]:** The extract was added with 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. It was transferred to a small test tube and

then 0.5 ml of conc. sulphuric acid was added carefully by the side of the test tube, blue colour appears in the acetic acid layer.

**5. Test for Saponin**

**a) Foam Froth Test:** Place 1ml of extract in water in a semi-micro tube and shaken well and noted for a stable froth.

**6. Test for Sterols**

**a) Salkowski test:** To the extract in Chloroform was added with few drops of conc. Sulfuric acid, shaken and allowed standing for some time, red colour appears at the lower layer indicated the presence of Steroids and formation of yellow coloured lower layer indicates the presence of triterpenoids

**7. Test for phenolic compounds**

2 ml of extract was treated with 2 ml of ferric chloride solution, Black precipitate indicates presence of phenol

**8. Test for Tannins**

**a) Gelatin test**: To the extract, 1% of gelatin and 10% sodium chloride were added a white precipitate formed.

**b) Lead acetate test:** The presence of white precipitate in the test solution, when treated with lead acetate solution indicates presence of tannins.

**9. Test for Flavonoids**

**a) Shinoda test (Magnesium Hydrochloride reduction test):** To the extract, few fragments of magnesium ribbon was added followed by conc. hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

**10. Test for Proteins & Amino Acids**

**a) Millon's test:** 0.5 ml of extract was treated with 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating.

**b) Ninhydrin test:** Few drops of extract was boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate), violet colour appears indicated the presence of Amino acids and Proteins.

**11. Test for Fats & Fixed Oils**

**a) Stain test:** The small quantity of extract was pressed between two filter papers; the stain on a filter paper indicates the presence of fixed oils.

**6.5 Chemical Analysis**

**(A).Tests for Acid radical**

**1. Test for Silicate**

A small amount of sample was shaken well with conc. HCl or Conc.$H_2SO_4$ – insoluble or sparingly soluble indicates the presence of silicates.

**2. Test for Phosphate**

2 ml of the extract was treated with 2 ml of ammonium molybdate solution and 2 ml of Conc.$HNO_3$ - Cloudy yellow appearance indicates the presence of phosphate.

**3. Test for Sulphate**

2 ml of the above prepared extract was added with 2 ml of diluted HCL, until the effervescence ceases off. Then 2 ml of Barium Chloride solution was added; white precipitate insoluble in con. HCL indicates the presence of sulphate.

**4. Test for Chloride**

2 ml of the above prepared extracts was added with 2 ml of dil- HNO3 till the effervescence ceases. Then 2ml of silver nitrate was added. Curdy white precipitate indicates the presence of chloride.

**5. Test for Sulphide**

To 1 g of the substance add 2 ml of con. HCL, Rotten egg smelling gas indicates the presence of sulphide.

**6. Test for Iodide**

To 2 ml of the above prepared extracts add dilute nitric acid and 0.5 ml of silver nitrate solution. Shake and allow to stand; a curdy, pale yellow precipitate indicates the presence of iodide.

**(B).Tests for Basic radical**

**1. Test for Iron**

To 1 ml of the above prepared extract add 1 ml of potassium ferrocyanide solution; an intense blue precipitate, insoluble in dilute hydrochloric acid indicates the presence of iron.

To 3 ml of the above prepared extract add 1 ml of 2 M hydrochloric acid and 1 ml of ammonium thiocyanate solution; the solution becomes blood-red in colour indicates the presence of iron.

**2. Test for Calcium**

To 2 ml of the extract add 2 ml of 4% ammonium oxalate solution, Cloudy appearance and white precipitate indicates the presence of calcium.

**3. Test for Sodium**

2 pinches (50 mg) of the sample was made into paste by using HCl and introduced into the blue flame of Bunsen burner, yellow colour flame indicates the presence of sodium.

**4. Test for Potassium**

A pinch (25 mg) of sample was treated with 2 ml of sodium nitrite solution and then treated with 2 ml of cobalt nitrate in 30% glacial acetic acid. Yellow precipitate indicates the presence of potassium.

**5. Test for Zinc**

5 ml of the extract was treated with 2 ml of potassium ferrocyanide solution; a white precipitate, insoluble in dilute hydrochloric acid indicates the presence of zinc.

**6. Test for Magnesium**

2 ml of the extract was treated with 1 ml of dilute ammonia solution; a white precipitate indicates the presence of magnesium.

**7. Test for Lead**

2ml of the extract was treated with 2ml of potassium iodide solution; yellow precipitate indicates the presence of lead.

## 6.6 *In vitro* antioxidant activity

### (i). DPPH radical scavenging assay [99]

**Principle**

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with red colour. Antioxidant reacts with DPPH and turns it to yellow. The degree of discolouration indicates the scavenging potentials of the antioxidant compounds.

$$(DPPH)+ (H\text{-}A) \longrightarrow DPPH\text{-}H + (A)$$

**Procedure**

DPPH radical scavenging assay of test sample was modified method described by Perumal *et al*., 2018. In brief, 0.135 mM DPPH was prepared in methanol. Different concentrations (5, 10, 20, 40, 80, 160 and 320 µg/ml) of sample was mixed with 2.5 ml of DPPH solution.

The reaction mixture was vortexed thoroughly and kept at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference standard.[100] The ability of extract to scavenge DPPH radical and control was calculated from the following formula

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where,

Abs control - Absorbance of the DPPH radical

Abs sample - Absorbance of DPPH + *Naga parpam*

The antioxidant activity of *Naga parpam* will be expressed as $IC_{50}$ and compared with the standard.

**(ii). Hydrogen peroxide scavenging assay [101]**

**Principle**

Among reactive oxygen species (ROS), hydrogen peroxide ($H_2O_2$), is a relatively stable, non-radical oxidizing species, may be formed in tissues through oxidative processes.

**Procedure**

The ability of the test sample to scavenge hydrogen peroxide is determined according to the method of Ruch et al (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentration of test samples (5, 10, 20, 40, 80, 160 and 320 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of the test samples and standard compound alpha-tocopherol was calculated

$$\% \text{ Scavenged} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where,

Abs control - Absorbance of the $H_2O_2$ radical

Abs sample - Absorbance of $H_2O_2$+ *Naga parpam*

The antioxidant activity of *Naga parpam* will be expressed as $IC_{50}$ and compared with the standard.

## 6.7 *In vivo* studies

### Experimental Animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/ IAEC approval no: **03/AEL/IAEC/MMC/2021-2022, Dated 21.10.2021.** Male Wistar albino rats used for this study were procured from Animal house, Madras Medical College, Chennai, India.

### Quarantine and Acclimatization

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. The newly procured male wistar albino rats were quarantined for a period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop the psychological, physiological and nutritional stabilization before their use.

### Housing

The animals were housed in well ventilated animal house which was maintained at constant temperature and relative humidity of 55 to 60%. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material. The bed material was changed twice a week**.**

### Diet and water

The animals were maintained on standard pellet and purified water. The animals were provided with food *ad libitum* except during fasting.

### Animal identification

All animal cages used in the study had a proper identification i.e., labels. Each animal in the cage was marked on tail with picric acid for their appropriate identification.

**Acute toxicity studies**

The acute toxicity study of *Naga parpam* has already been done using male albino rats of wistar strain (180-200gm) and it was shown to be non toxic at the dose of 40 mg/kg orally. Thus 1/10th and 1/5th of maximum dose, 4 mg/kg and 8 mg/kg will be used in this study. [104]

**Grouping of animals**

Totally 24 male albino rats was used in this study. The animals were divided into 4 groups, each group containing 6 animals.

**Table no .3. Experimental design**

| S.NO | GROUP (n=6) | TREATMENT SCHEDULE |
|---|---|---|
| 1 | Normal Control | 1% w/v Carboxymethyl cellulose (CMC) |
| 2 | Disease Control | 1.5mg/kg of MCP orally daily for 45 days [11] |
| 3 | Insecticide + Low dose of *Naga parpam* | 1.5mg/kg of MCP orally daily for 45 days + 4 mg/kg of *Naga parpam* p.o for 45 Days |
| 4 | Insecticide + High dose of *Naga parpam* | 1.5mg/kg of MCP orally for 45 days + 8 mg/kg of *Naga parpam* p.o for 45 Days |

**Necropsy**

After completion of the experimental period (45 days), the rats were fasted overnight. On the 46th day, the rats were weighed and euthanized using light anesthesia of Isoflurane. For serum testosterone estimation, the blood was collected by cardiac puncture in non-heparinized tubes for serum separation, centrifuged at 1400 x g for 15 min using Remi C 854/8 centrifuge, and kept at - 20 ◦C until assessing.

## 6.8 Evaluation parameters

### 1. Reproductive organ weight

Testes, epididymis, seminal vesicles and prostate gland were quickly removed, washed with ice-cold 0.9 % NaCl and weighed separately for the nearest milligram immediately using electronic balance after clearing off the adhering tissues.

### 2. Sperm Parameters

**Epididymal sperm analysis** [105]

Sperm parameters are the valuable indicators of male fertility. In this study, the sperm count, viable sperm, and motile sperm from the cauda part of the epididymis were analysed to assess the extent of insecticide toxicity and also the protective efficacy of the *Naga parpam* on these sperm variables.

The evaluation of sperm motility was performed according to the method described by Belsey *et al*. The cauda epididymis was minced in physiological saline (0.9% NaCl in distilled water) at 37°C, and the resultant epididymal fluid was used to analyze the selected sperm variables. To analyze the percent motile sperm, the diluted epididymal fluid was placed in Neubauer hemocytometer and total, motile, and non-motile sperms are counted. The number of motile and non-motile sperms was determined microscopically within 5 min following their isolation from the cauda epididymis at 37°C.

For the analysis of the live and dead sperms, trypan blue reagent method was used in this study. Briefly, one drop of the diluted epididymal sperm suspension was mixed with one drop of eosin and five drops of nigrosin solution and incubated at 37°C for 15 min. The mixture was placed on a microscope slide, covered with a cover slip and observed under a microscope. Sperms were considered as viable if they were unstained and considered as dead if they were stained with the eosin and appears red in colour.

The units for sperm count, viable sperm, and motile sperm were expressed as millions/ml, percentage of total non-motile sperm of the total sperm and percentage of total unstained sperm of the total sperm, respectively.

**3. Estimation of Lipid Peroxidation in Testes** [105]

**PRINCIPLE**

MDA is an end product of lipid peroxidation that react with TBA to gives a pink colour. Absorbance measured at 532 nm.

**PROCEURE**

Determination of lipid peroxidation levels in terms of Thiobarbituric acid reactive substances (TBARS) provides valuable information about the cellular/tissue oxidative stress. In this study, the levels of TBARS was analyzed spectrophotometrically in the testis of controls and experimental rats based on the method described by Ohkawa *et al*. The testis (10% W/V) was homogenized in homogenization buffer containing 1.15% KCl solution. To the homogenate (2.5 ml), 0.5 ml of saline (0.9% sodium chloride) and 1.0 ml of (20% W/V) Trichloroacetic acid was added and centrifuged for 20 min at 4000 rpm. To the resultant supernatant (1.0 ml), 0.25 ml of TBA reagent was added and the mixture was incubated at 95°C for 1 h. Finally, an equal volume of n-butanol was added to the mixture and the contents will be centrifuged for 15 min at 4000 rpm. The organic layer was carefully transferred into a clear tube, and its absorbance will be measured Spectrophotometrically at 532 nm. The absorbance was recorded and compared against the standard curve constructed with the known amount of Malondialdehyde. The rate of lipid peroxidation was expressed as μ moles of Malondialdehyde formed/gram wet weight of tissue.

**4. Assay of antioxidant enzymes in testes** [105]

**GENERAL PROCEDURE FOR HOMOGENIZATION**

The testis was homogenized (10% w/v) in 50 mM ice-cold sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA. Then the homogenate was centrifuged for 60 minutes. The supernatant was used for the estimation of antioxidant enzyme levels.

**A. SUPER OXIDE DISMUTASE**

The activity of SOD was determined by the method of Mishra and Fridovich. SOD was assayed in the microsomal fraction according to its ability to inhibit the auto-oxidation of epinephrine at alkaline medium. The reaction mixture contained 0.05 M carbonate buffer (pH 10.2), 30 mM epinephrine (freshly prepared) and the tissue homogenate. Changes in absorbance

was recorded at 480 nm and measured at 10s intervals for 1 min in a spectrophotometer. The enzyme activity was expressed as units/mg protein/min.

**B. CATALASE**

The activity of CAT was determined by based on its ability to decompose hydrogen peroxide ($H_2O_2$), from the method of Chance and Machly. The reaction mixture in a volume of 2.5 ml contained 0.05M phosphate buffer (pH 7.0), 19 mM $H_2O_2$, and appropriate amount of tissue homogenate. Then, the absorbance was read at 240 nm and measured at 10 s intervals for 1 min in a spectrophotometer. CAT activity was expressed as μM of $H_2O_2$ metabolized/mg protein/min.

**C. GLUTATHIONE PEROXIDASE**

The activity of GPx was estimated by the method of Mohandas *et al*. Briefly, the reaction mixture contained 1.59 ml of 100 mM phosphate buffer (pH 7.6), 0.1 ml of 10 mM EDTA, 0.1 mL of 10 mM sodium azide, 0.1 ml glutathione reduced, 0.01 ml of 0.2 mM hydrogen peroxide, and 0.1 ml of tissue homogenate. Immediately, the contents was read at 340 nm against blank, at 10 s intervals for 3 min on a spectrophotometer. The activity of GPx was expressed as nanomoles of NADPH oxidized/mg protein/min.

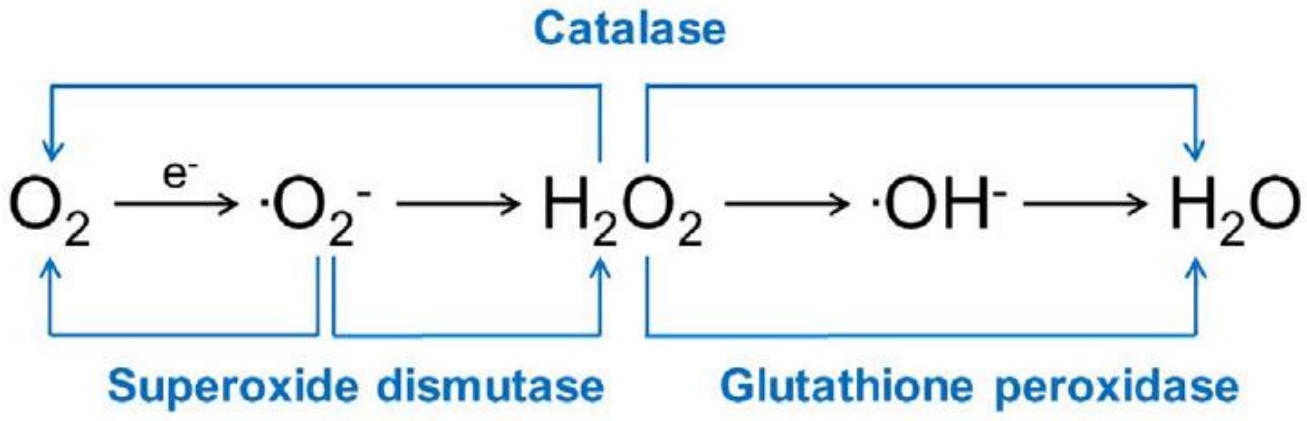



**Figure: 9. Enzymatic antioxidants that react with and buffer free radicals.**

**5. Estimation of Serum Testosterone level**

Serum testosterone level was assayed using ECLIA (Electrochemiluminescence immunoassay) method based on the principle of competitive binding and according to the manufacturer's instructions.

**6. Histopathological analysis**

A single testis was isolated from each rat in control, Monocrotophos and *Naga parpam* treated groups and then fixed in 10 % neutral buffered formalin for 24 h. The fixer was washed with running tap water overnight. The tissues were cleaned with methyl benzoate after drying using a graded series of alcohols and embedded in wax with paraffin. At 6 m thickness, testis sections were cut, stained with hematoxylin and eosin dissolved in 95 % Ethanol was used to stain the counter. Testis sections were observed under a microscope after dehydration and clearing.

**STATISTICAL ANALYSIS**

Data are presented as mean ± SEM and the values of $P < 0.01$ were considered statistically significant. Statistical analysis between the control and experimental groups was analyzed using one-way analysis of variance followed by Dunnett's multiple comparison test using Graph Pad Prism, version (8.0.2).

# RESULTS

# 7. RESULTS

## 7.1. ORGANOLEPTIC CHARACTERS

The *Naga parpam* was subjected to various organoleptic tests and the results were determined and illustrated in **Table no.4.**

**Table no. 4. Organoleptic characters of *Naga parpam***

| S.NO | CHARACTER | OBSERVATION |
|---|---|---|
| 1. | Colour | Yellowish white |
| 2. | Odour | Odourless |
| 3. | Texture | Fine powder |
| 4. | Taste | Tasteless |

## 7.2. PHYSICOCHEMICAL ANALYSIS

The *Naga parpam* was evaluated for physicochemical analysis and the results were determined and illustrated in **Table no.5.**

**Table no. 5. Physicochemical analysis of *Naga parpam***

| S.NO | PARAMETERS | RESULT |
|---|---|---|
| 1. | pH | 8.05 |
| 2. | Loss on drying | 0.5 % |
| 3. | Total Ash value | 97.81 % |
| 4. | Acid insoluble ash | 5.58 % |
| 5. | Water soluble extractive | Less than 1 % |
| 6. | Alcohol soluble extractive | Less than 1 % |

## 7.3. PHYTOCHEMICAL ANALYSIS

The aqueous extract of *Naga parpam* was evaluated for phytochemical screening and the results are illustrated in **Table no.6.**

**Table no. 6. Phytochemical analysis of *Naga parpam***

| S.NO | PHYTOCHEMICAL | TEST | OBSERVATION |
|---|---|---|---|
| 1. | Alkaloids | Mayer's Test | Negative |
| | | Wagner's Test | |
| | | Dragendroff's Test | |
| | | Hager's Test | |
| 2. | Carbohydrates | Molisch's Test | Negative |
| | | Benedict's Test | |
| 3. | Glycoside | Legal's Test | Negative |
| | | Keller Killiani Test | |
| 4. | Saponin | Froth Test | Negative |
| | | Foam Test | |
| 5. | Phytosterol | Salkowski's Test | Negative |
| 6. | Phenols | Ferric Chloride Test | Negative |
| 7. | Tannins | Gelatin Test | Negative |
| 8. | Flavonoids | Shinoda test | Negative |
| 9. | Amino acid | Millon's test | Negative |
| | | Ninhydrin test | |
| 10. | Fats & Fixed Oils | Stain test | Negative |

## 7.4. CHEMICAL ANALYSIS

The *Naga parpam* was evaluated for the presence of various acid and basic radicals and the results are illustrated in **Table no. 7 and Table no.8.**

**Table no. 7. Test for acid radicals**

| S.No | Experiement | Observation | Inference |
|---|---|---|---|
| 1. | **Test for Silicate** | Sparingly soluble | **Presence of Silicate** |
| 2. | **Test for Phosphate** | No Yellow Precipitation | Absence of Phosphate |
| 3. | **Test for Sulphate** | No White Precipitation | Absence of Sulphate |
| 4. | **Test for Chloride** | Cloudy White Precipitation | **Presence of Chloride** |
| 5. | **Test for Sulphide** | No rotten egg smell | Absence of Sulphide |
| 6. | **Test for Iodide** | No Yellow Precipitate | Absence of Iodide |

**Table no. 8. Test for basic radicals**

| S.No | Experiement | Observation | Inference |
|---|---|---|---|
| 1. | **Test for Iron** | Red Colour | **Presence of Iron** |
| 2.. | **Test for Calcium** | No White Precipitation | Absence of Calcium |
| 3.. | **Test for Sodium** | No Yellow Flame | Absence of Sodium |
| 4. | **Test for Potassium** | Yellow Precipitation | **Presence of Potassium** |
| 5. | **Test for Zinc** | White Precipitation | **Presence of Zinc** |
| 6. | **Test for Magnesium** | No White Precipitation | Absence of Magnesium |
| 7. | **Test for Lead** | No Yellow Precipitation | Absence of Lead |

## 7.5 *IN VITRO* ANTIOXIDANT ACTIVITY OF NAGA PARPAM

### 7.5.1. DPPH radical scavenging activity

In vitro Anti-oxidant activity of *Naga parpam* was evaluated by DPPH assay. The results obtained are illustrated in **Table no.9 and Figure 10.**

**Table no. 9. DPPH free radical scavenging assay**

| S.No | Concentration (µg/ml) | Standard (Ascorbic acid) | | Test Drug (*Naga parpam*) | |
|---|---|---|---|---|---|
| | | % Inhibition | IC $_{50}$ Value | % Inhibition | IC $_{50}$ Value |
| 1 | 5 | 13.20 ± 0.33 | **23.82 µg/ml** | 2.69 ± 0.40 | **83.73 µg/ml** |
| 2 | **10** | 33.19 ± 0.33 | | 12.09 ± 0.33 | |
| 3 | **20** | 49.16 ± 0.40 | | 23.86 ± 0.40 | |
| 4 | **40** | 66.33 ± 0.40 | | 35.79 ± 0.33 | |
| 5 | **80** | 78.11 ± 0.33 | | 46.36 ± 0.46 | |
| 6 | **160** | 87.60 ± 0.40 | | 66.34 ± 0.48 | |
| 7 | **320** | 92.85 ± 0.48 | | 75.42 ± 0.60 | |

**All the values are expressed in mean ± SEM (n=3), All the values are significant (P < 0.05) when compared to control and standard.**

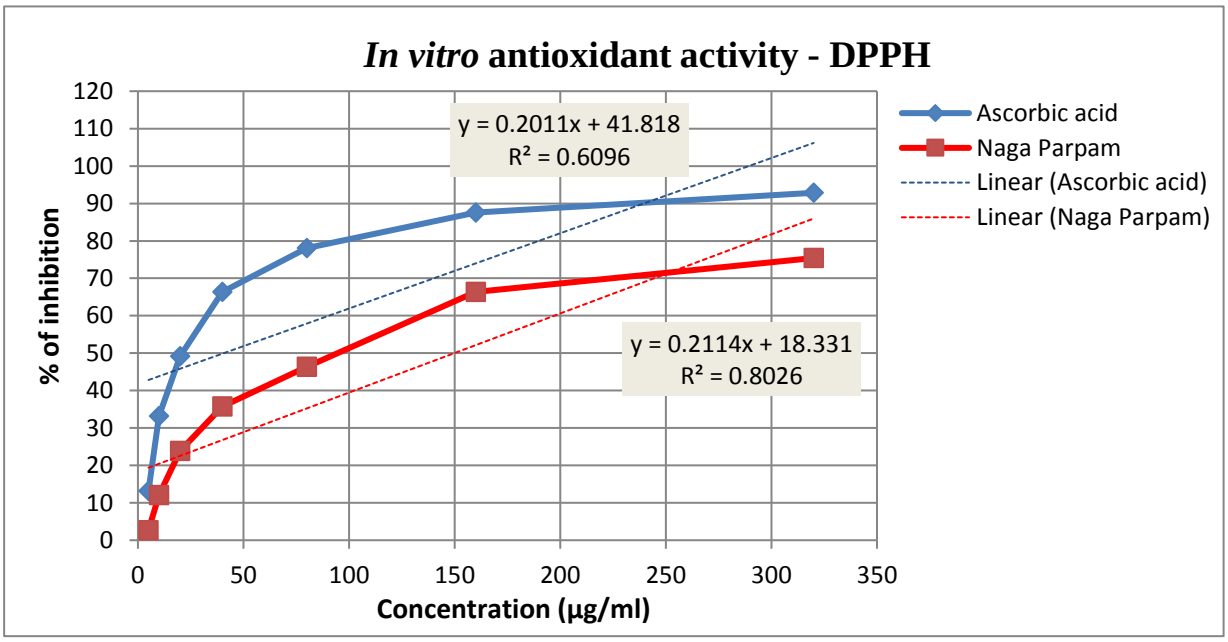



**Figure: 10. Effect of *Naga parpam* on DPPH radical scavenging activity**

From the results the IC50 value of *Naga parpam* was found to be **83.73 µg/ml** which is comparable to that of standard Ascorbic acid which the $IC_{50}$ value was found to be **23.82 µg/ml**

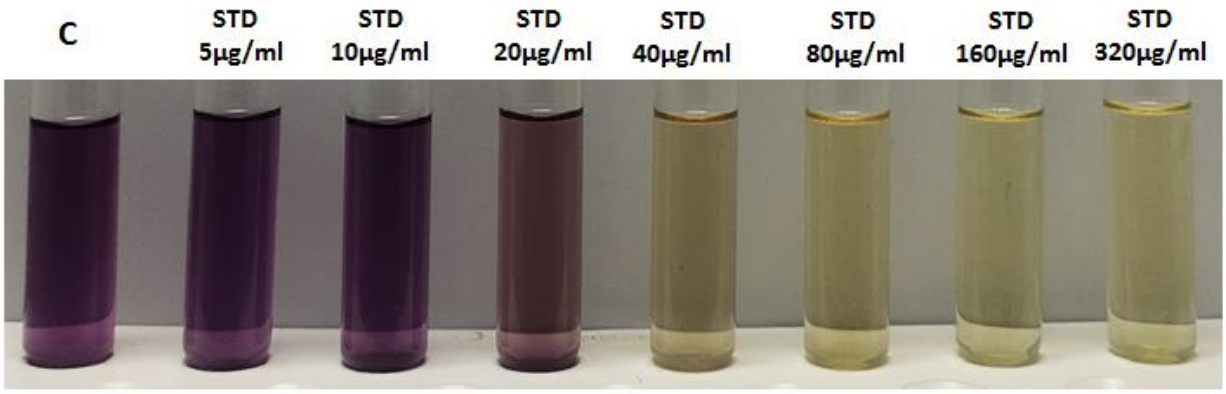



**Figure: 11. DPPH radical scavenging activity-Standard ascorbic acid**

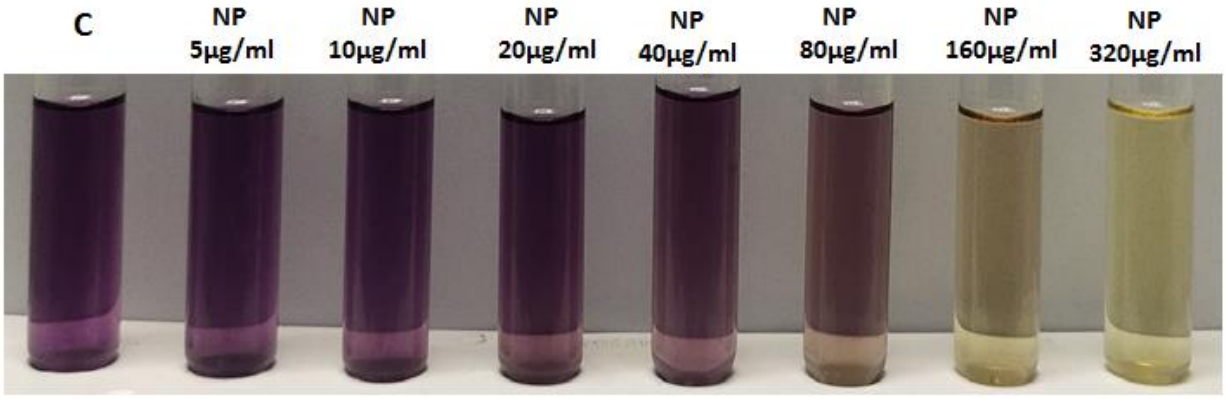



**Figure: 12. DPPH radical scavenging activity-*Naga parpam***

### 7.5.2. Hydrogen peroxide scavenging assay

In vitro Anti-oxidant activity of *Naga parpam* was evaluated by Hydrogen peroxide scavenging assay. The results obtained are illustrated in **Table no.10 and Figure 12.**

**Table no. 10. Hydrogen peroxide scavenging assay**

| S.No | Concentration (µg/ml) | Standard (Alpha tocopherol) | | Test Drug (*Naga parpam*) | |
|---|---|---|---|---|---|
| | | % Inhibition | IC $_{50}$ Value | % Inhibition | IC $_{50}$ Value |
| 1 | 5 | 17.24 ± 0.13 | **24.06 µg/ml** | 2.54 ± 0.26 | **106.9 µg/ml** |
| 2 | **10** | 22.52 ± 0.26 | | 6.96 ± 0.35 | |
| 3 | **20** | 41.67 ± 0.13 | | 15.97 ± 0.35 | |
| 4 | **40** | 71.93 ± 0.26 | | 25.32 ± 0.35 | |
| 5 | **80** | 84.09 ± 0.13 | | 43.81 ± 0.30 | |
| 6 | **160** | 93.65 ± 0.13 | | 62.17 ± 0.30 | |
| 7 | **320** | 96.22± 0.18 | | 72.97 ± 0.26 | |

**All the values are expressed in mean ± SEM (n=3), All the values are significant (P < 0.05) when compared to control and standard.**

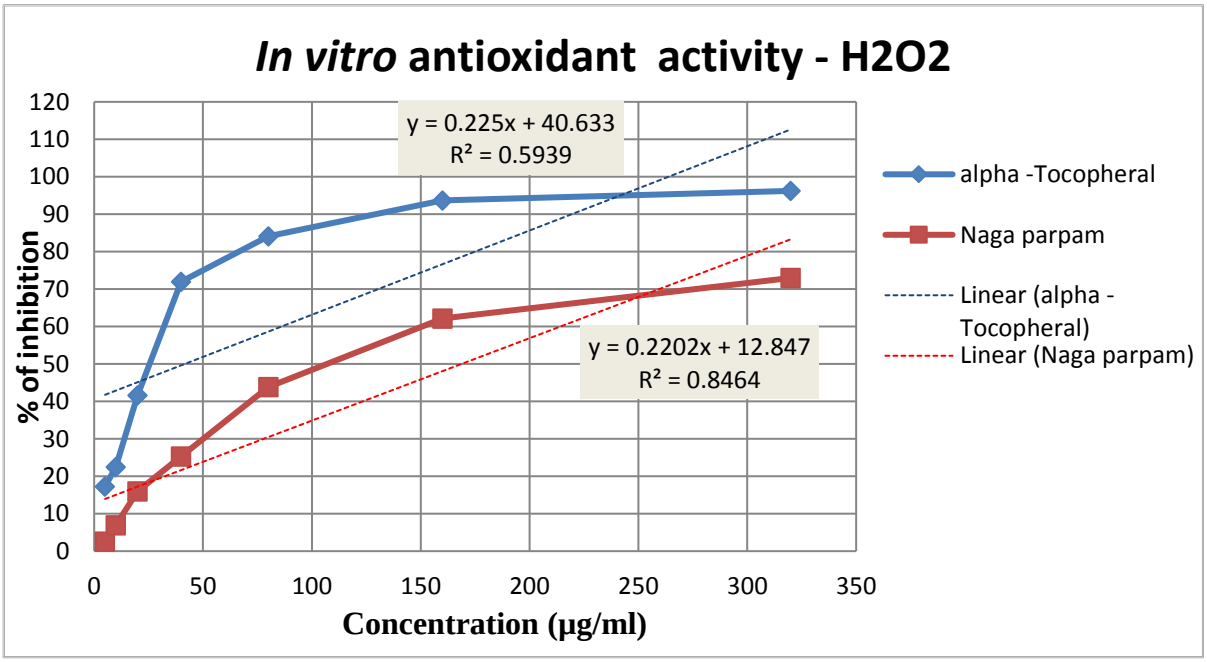



**Figure: 13. Effect of *Naga parpam* on Hydrogen peroxide scavenging assay**

From the results the $IC_{50}$ value of *Naga parpam* was found to be **106.9 µg/ml** which is comparable to that of standard α- tocopherol which the $IC_{50}$ value was found to be **24.06 µg/ml.**

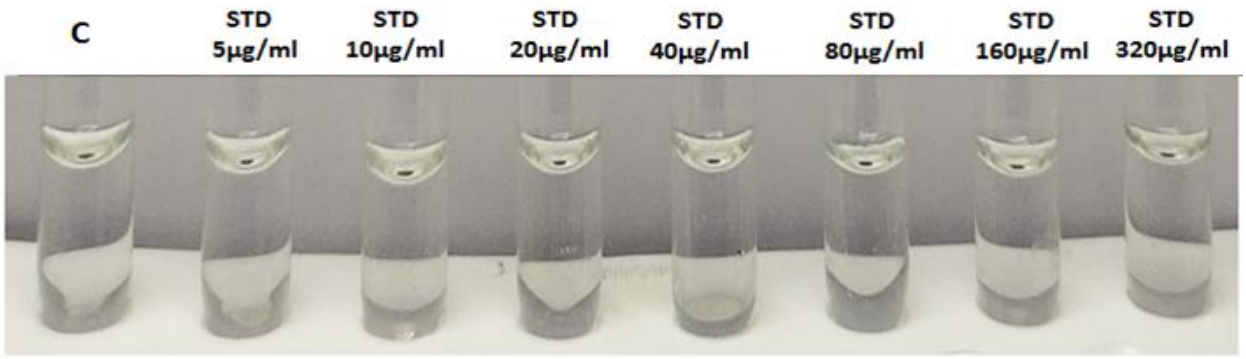



**Figure: 14. Hydrogen peroxide scavenging assay- α Tocopherol**

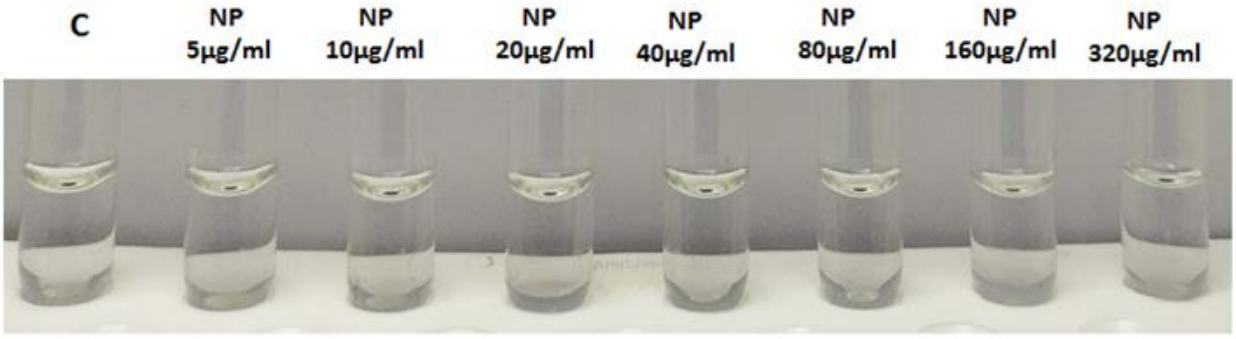



**Figure: 15. Hydrogen peroxide scavenging assay- *Naga parpam***

## 7.6 *IN VIVO* ANTI-INFERTILITY ACTIVITY OF *NAGA PARPAM*

### 7.6.1. Effect of *Naga parpam* on body weight

The body weight of animals was determined on day 0 and 46$^{th}$ day of the study period and they are illustrated in **Table no. 11** and **Figure 16.**

During the experiment, some signs of toxicity were noticed such as huddling, mild tremor, diarrhea and food avoidance but no mortality occurred in all the exposed animals. This may be related to cholinergic signs, a consistent sign in organophosphate poisoning. On the other hand, such clinical signs were not noticed in normal control rats.

**Table no. 11. Body weight variation**

| S.No | Group | Body Weight (g) | | | |
|---|---|---|---|---|---|
| | | Initial | Final | Difference | Weight Change (%) |
| 1 | Normal Control | 186.5 ± 4.54 | 237.5 ± 5.43 | 51 | 27.35 |
| 2 | Disease Control | 206 ± 3.00 | 223.33 ± 5.73 | 17.33 | 8.41 |
| 3 | Low Dose – *Naga parpam* | 210.75 ± 10.5 | 234.75 ± 8.20 | 24 | 11.39 |
| 4 | High Dose – *Naga parpam* | 214.75 ± 1.98 | 218.5 ± 2.72 | 3.75 | 1.75 |

**All the values are expressed as Mean ± SEM. (n=6)**

The body weight of MCP exposed rats was increased by **8.41%** which is significantly lower when compared to normal control rats whose body weight increased to **27.35%**. The percentage increase in body weight of low dose-NP exposed group was **11.39 %** which is higher than MCP exposed rats whereas high dose – NP exposed rats only gained **1.75 %** which is lower than both normal and MCP exposed rats.

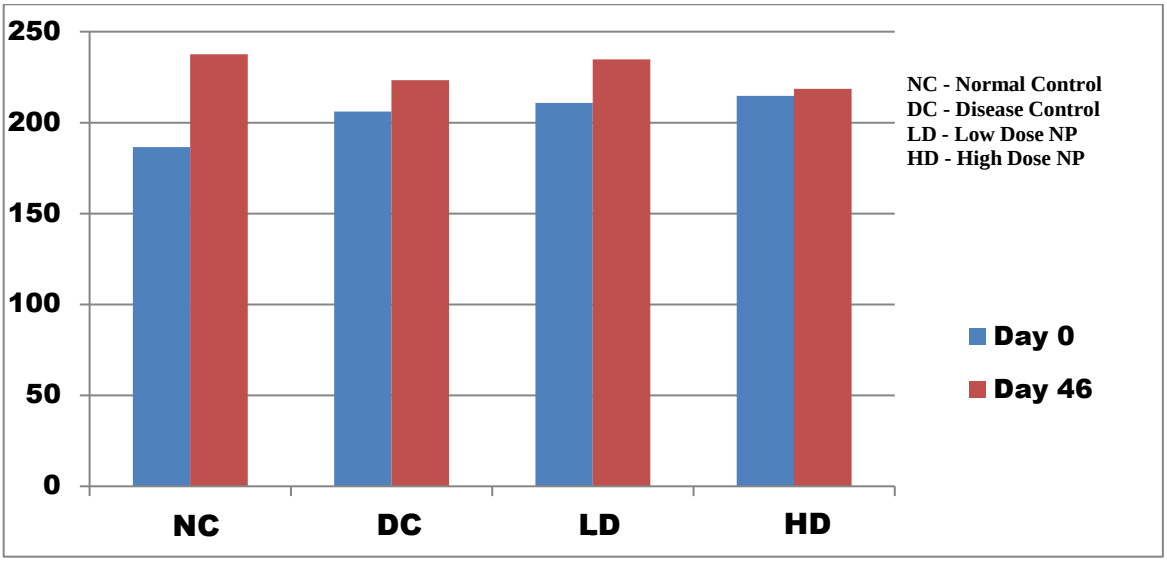



**Figure: 16. Body weight variation**

### 7.6.2. Effect of *Naga parpam* on male reproductive organs

The effect of *Naga parpam* and MCP on weight of testis was determined and illustrated in **Table no .12** and **Figure 17.**

**Table no. 12. Effect of MCP and NP on weight of testis**

| S.No | Group | Weight of Testis (g) |
|---|---|---|
| 1 | Normal Control | 3.83 ± 0.18 |
| 2 | Disease Control | 1.04 ± 0.04 |
| 3 | Low Dose – *Naga parpam* | 2.43 ± 0.13 |
| 4 | High Dose – *Naga parpam* | 1.72 ± 0.14 |

**All the values are expressed as Mean ± SEM. (n=6), $^{###}$P<0.001 compared to normal control group, $^{***}$P<0.001 compared to disease control group, $^{**}$ P<0.01 compared to disease control group, $^{*}$ P<0.05 compared to disease control group**

The effect of Monocrotophos and *Naga parpam* on weight of the testis was determined and tabulated in **Table no.12** and **Figure 17**. There was a significant reduction (P<0.001) in testicular weight in rats exposed to MCP alone when compare to normal control rats which was

significantly increased by *Naga parpam.* Among both low dose and high dose groups, low dose improves the testicular weight more effectively than high dose group.

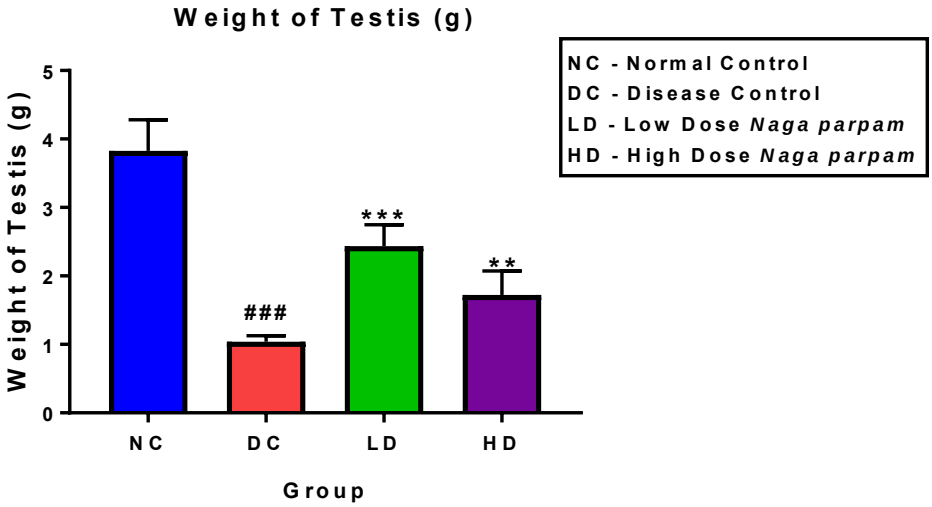



**Figure: 17. Weight of Testis**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group, ** P<0.01 compared to disease control group**

**Table no. 13. Effect of MCP and NP on weight of Seminal vesicles**

| S.No | Group | Weight of Seminal vesicles (g) |
|---|---|---|
| 1 | Normal Control | 1.09 ± 0.08 |
| 2 | Disease Control | 0.48 ± 0.02 |
| 3 | Low Dose – *Naga parpam* | 0.87 ± 0.02 |
| 4 | High Dose – *Naga parpam* | 0.65 ± 0.02 |

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group, * P<0.05 compared to disease control group**

The effect of Monocrotophos and *Naga parpam* on weight of the seminal vesicles was determined and illustrated in **Table no.13** and **Figure 18**. There was a significant reduction (P<0.001) in seminal vesicles weight in rats exposed to MCP alone when compare to normal control rats which was significantly increased by *Naga parpam.* Among both low dose and high dose groups, low dose improves the seminal vesicles weight more effectively than high dose group.

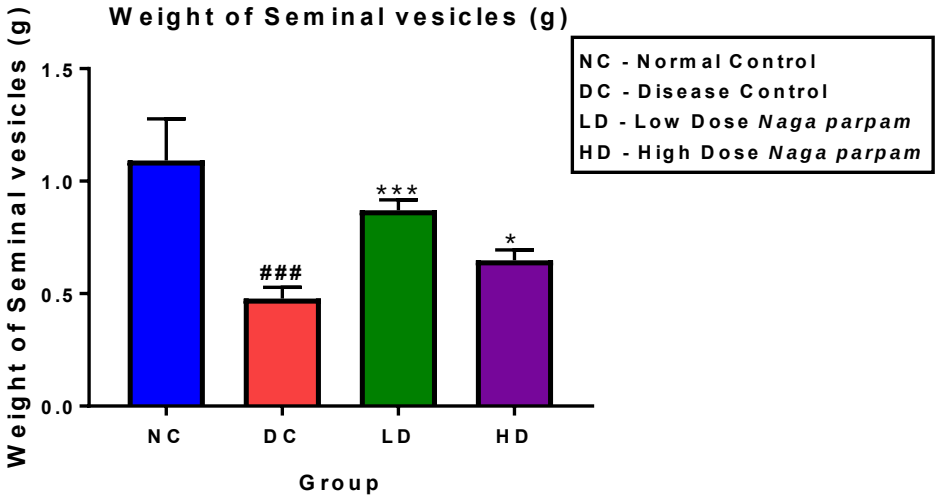



**Figure: 18. Weight of Seminal vesicles**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group, * P<0.05 compared to disease control group**

**Table no. 14. Effect of MCP and NP on weight of Prostate gland**

| S.No | Group | Weight of Prostate gland (g) |
|---|---|---|
| 1 | Normal Control | 1.21 ± 0.01 |
| 2 | Disease Control | 0.25 ± 0.03 |
| 3 | Low Dose – *Naga parpam* | 0.55 ± 0.02 |
| 4 | High Dose – *Naga parpam* | 0.45 ± 0.01 |

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

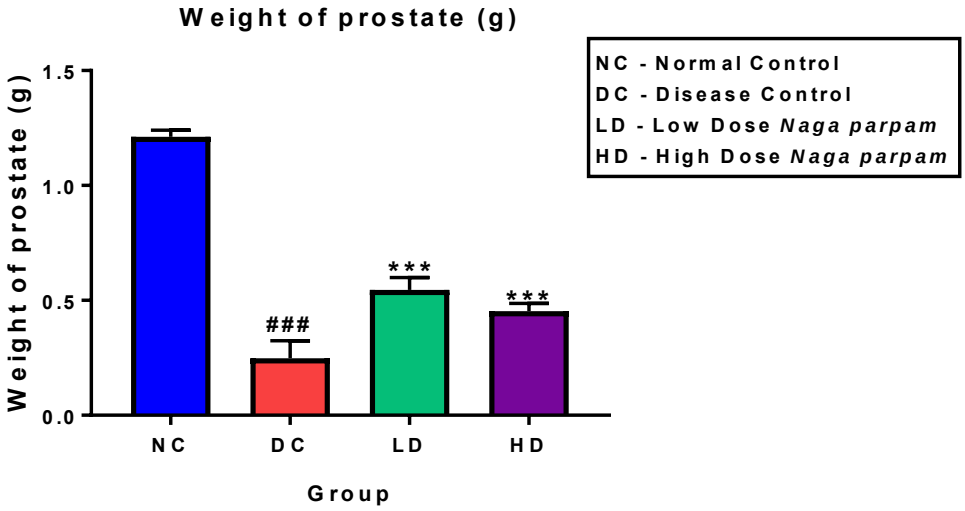



**Figure: 19. Weight of prostate**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

The effect of Monocrotophos and *Naga parpam* on weight of the prostate was determined and illustrated in **Table no.14** and **Figure 19.** There was a significant reduction (P<0.001) in prostate weight in rats exposed to MCP alone when compare to normal control rats which was significantly increased by *Naga parpam.* Among both low dose and high dose groups, low dose improves the prostate weight more effectively than high dose group.

**Table no. 15. Effect of MCP and NP on weight of Epididymis**

| S.No | Group | Weight of Epididymis (g) |
|---|---|---|
| 1 | Normal Control | 0.69 ± 0.02 |
| 2 | Disease Control | 0.40 ± 0.02 |
| 3 | Low Dose – *Naga parpam* | 0.61 ± 0.01 |
| 4 | High Dose – *Naga parpam* | 0.56 ± 0.01 |

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

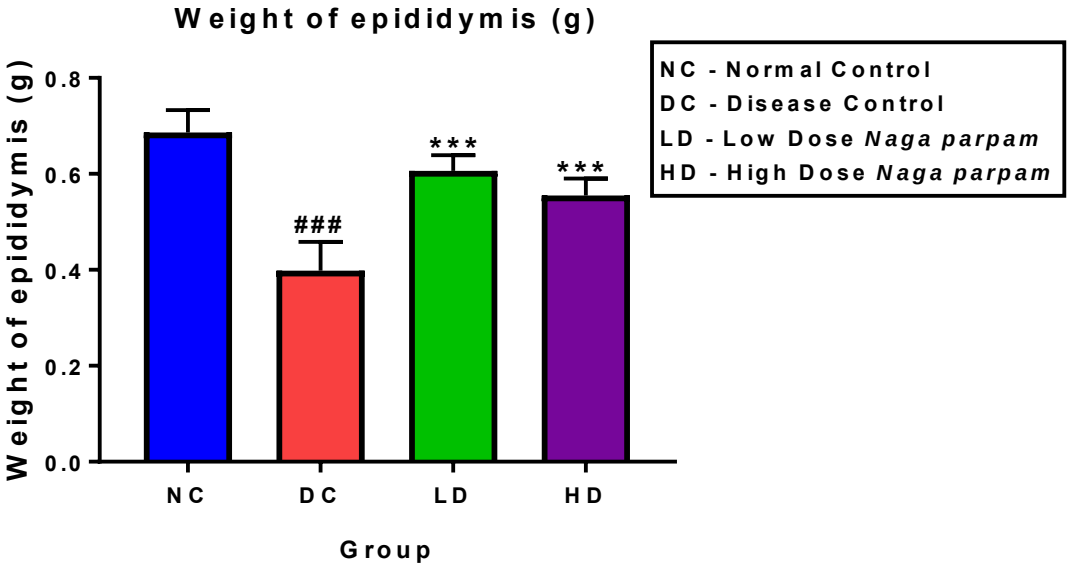



**Figure: 20. Weight of epididymis (g)**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

The effect of Monocrotophos and *Naga parpam* on weight of the epididymis was determined and illustrated in **Table no.15** and **Figure 20.** There was a significant reduction (P<0.001) in epididymis weight in rats exposed to MCP alone when compare to normal control rats which was significantly increased by *Naga parpam.* Among both low dose and high dose groups, low dose improves the epididymis weight more effectively than high dose group.

### 7.6.3. Evaluation of sperm parameters

**Table no. 16. Evaluation of sperm parameters**

| S.No | Group | Sperm characteristics | | |
|---|---|---|---|---|
| | | Count (x10 $^{6}$) | Motility (%) | Viability (%) |
| 1 | Normal Control | 131 ± 0.01 | 78.6 ± 1.43 | 55.65 ± 1.41 |
| 2 | Disease Control | 65 ± 1.26 $^{a}$ | 34.7 ± 1.43 $^{a}$ | 26.61 ± 1.29 $^{a}$ |
| 3 | Low Dose – *Naga parpam* | 105 ± 1.43 $^{b}$ | 51.5 ± 1.86 $^{b}$ | 45.39 ± 1.32 $^{b}$ |
| 4 | High Dose – *Naga parpam* | 90 ± 1.09$^{b}$ | 53.8 ± 1.24 $^{b}$ | 36.92 ± 0.79 $^{b}$ |

**All the values are expressed as Mean ± SEM. (n=6), $^{a}$ P<0.001 compared to normal control group, $^{b}$ P<0.001 compared to disease control group**

The epididymal sperm count was determined and results are illustrated in **Table no .16 and Figure 21**. The sperm count in normal male rats was found to be **131 ± 0.01 Millions/ml**. The rats treated with Monocrotophos showed a highly significant reduction (P<0.001) in the sperm count after 45 days treatment. The sperm count was found to be only **65 ± 1.26 millions/ml**. The rats which were treated with *Naga parpam* showed significant (P< 0.001) improvement in the sperm count. At the lower dose of 4 mg/kg, the sperm count increased to **105 ± 1.43 millions/ml**. This count however is still significantly lesser (P<0.001) than the normal count. But at the higher dose of 8 mg/kg, the *Naga parpam* was able to increase the sperm count to **90 ± 1.09 millions/ml** and this count was even marginally lower than the low dose value.

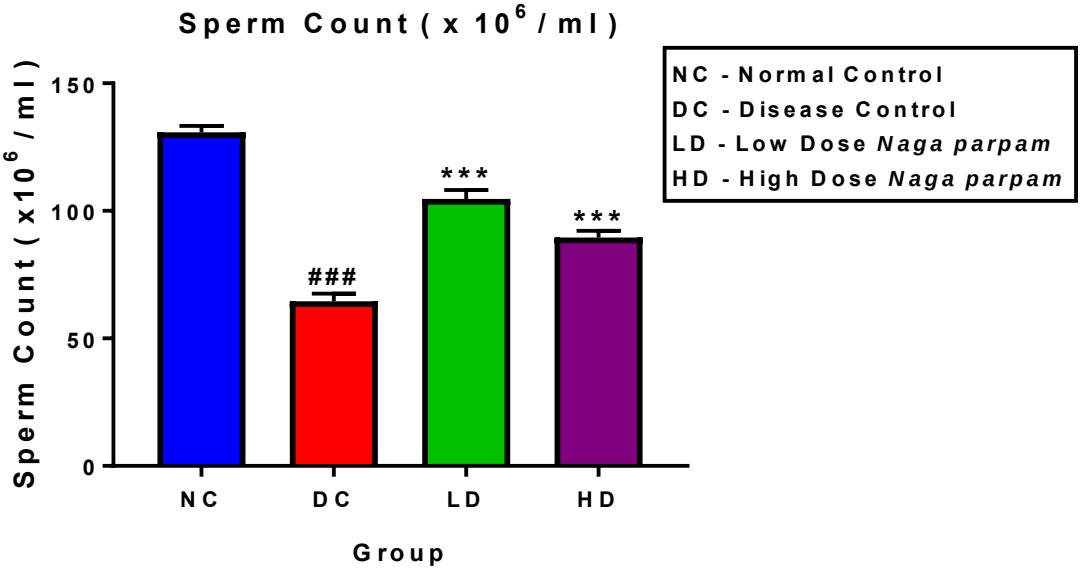



**Figure: 21. Effect of MCP and NP on Sperm count**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

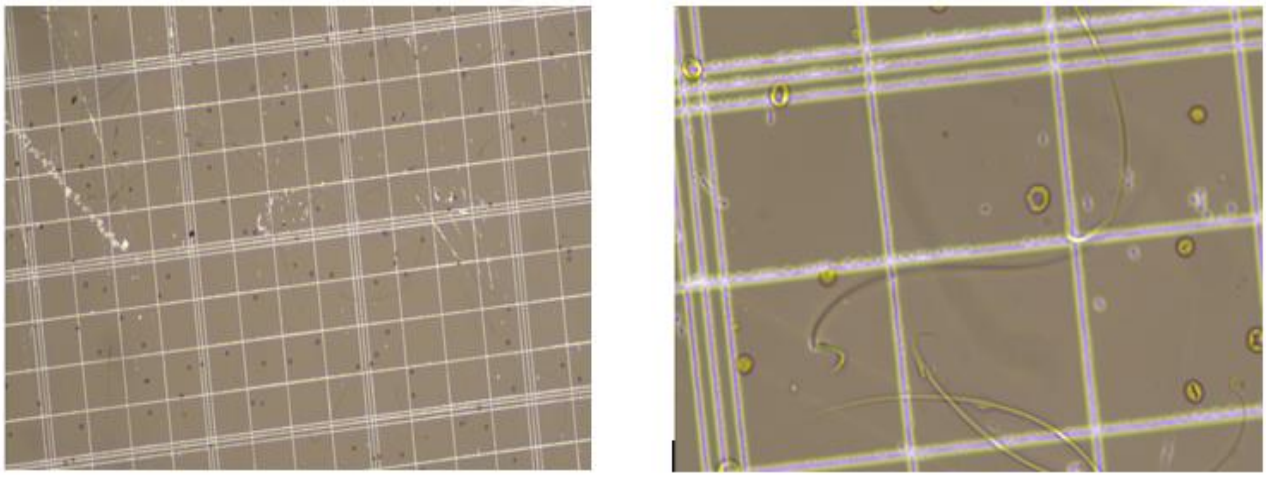


**Figure: 22. Sperm count in Neubauer Hemocytometer.**

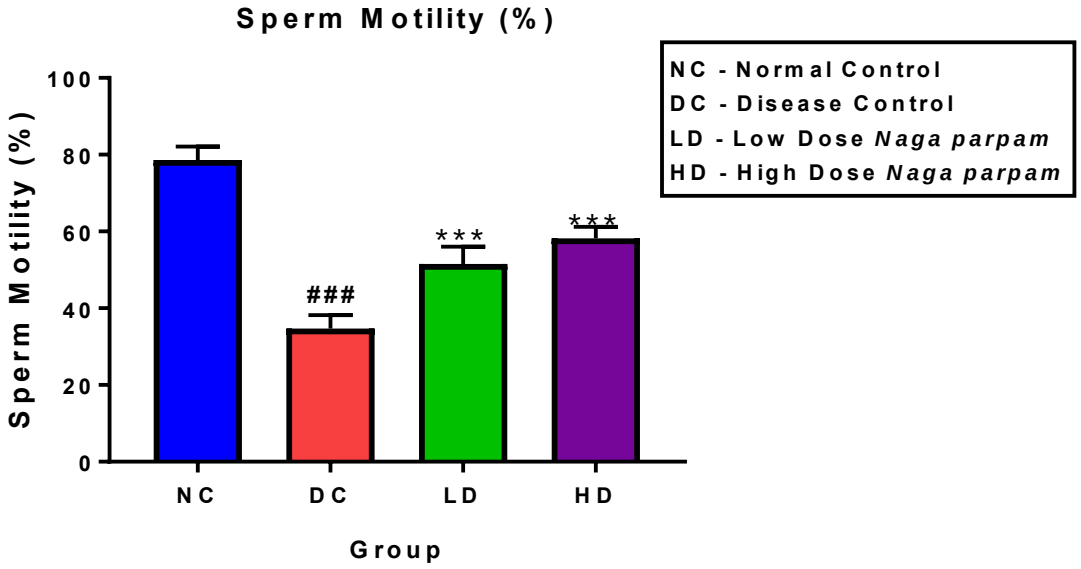



**Figure: 23. Effect of MCP and NP on Sperm motility**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

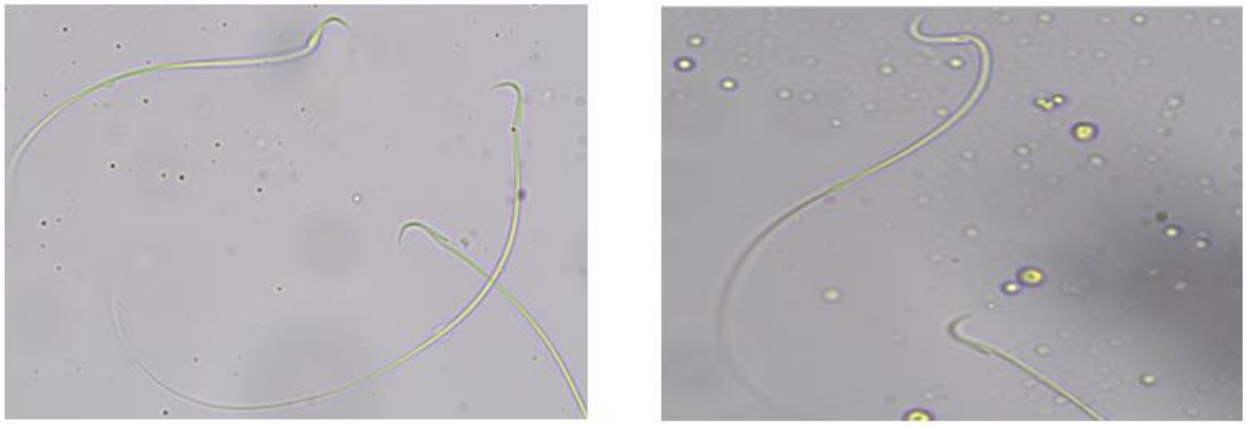


**Figure: 24. Microscopical image of rat sperm**

The epididymal sperm motility was determined and results are illustrated in **Table no.16 and Figure 23.** The sperm motility in normal male rats was found to be **78.6 ± 1.43 %**. The rats treated with Monocrotophos showed a highly significant (P<0.001) reduction in the sperm motility after 45 days treatment. The sperm motility was found to be **34.7 ± 1.43 %**. In the rats which were treated with *Naga parpam* showed a significant (P<0.001) improvement in the sperm motility. At the lower dose of 4 mg/kg, the sperm motility was increased to **51.5 ± 1.86 %**. This

motility however is still significantly (P<0.001) lesser than the normal motility. At the higher dose of 8 mg/kg, the *Naga parpam* was able to increase the sperm motility to **53.8 ± 1.24 %** which is slightly higher than the low dose value.

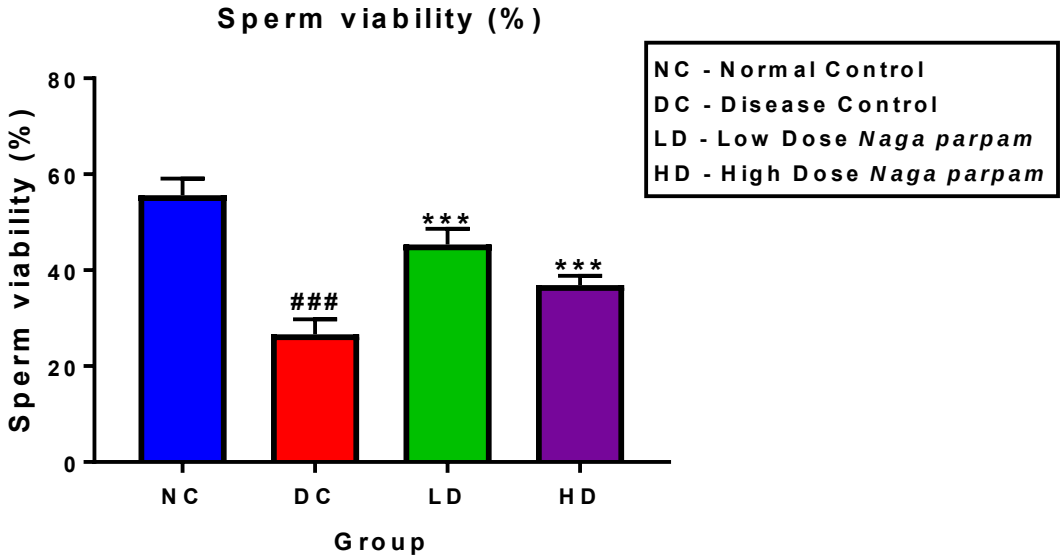



**Figure: 25. Effect of MCP and NP on Sperm viability**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

The epididymal sperm viability was determined and results are illustrated in **Table no. 16 and Figure 25.** The sperm viability in normal male rats was found to be **55.65 ± 1.41 %**. The rats treated with Monocrotophos showed a highly significant reduction (P<0.001) in the sperm viability after 45 days treatment. The sperm viability was found to be **26.61 ± 1.29 %.** In the rats which were treated with *Naga parpam* showed a significant (P<0.001) improvement in the sperm viability. At the lower dose of 4 mg/kg, the sperm viability was increased to **45.39 ± 1.32 %**. This motility however is still significantly lesser (P<0.001) than the normal viability. At the higher dose of 8 mg/kg, the *Naga parpam* was able to increase the sperm viability to **36.92 ± 0.79 %**.

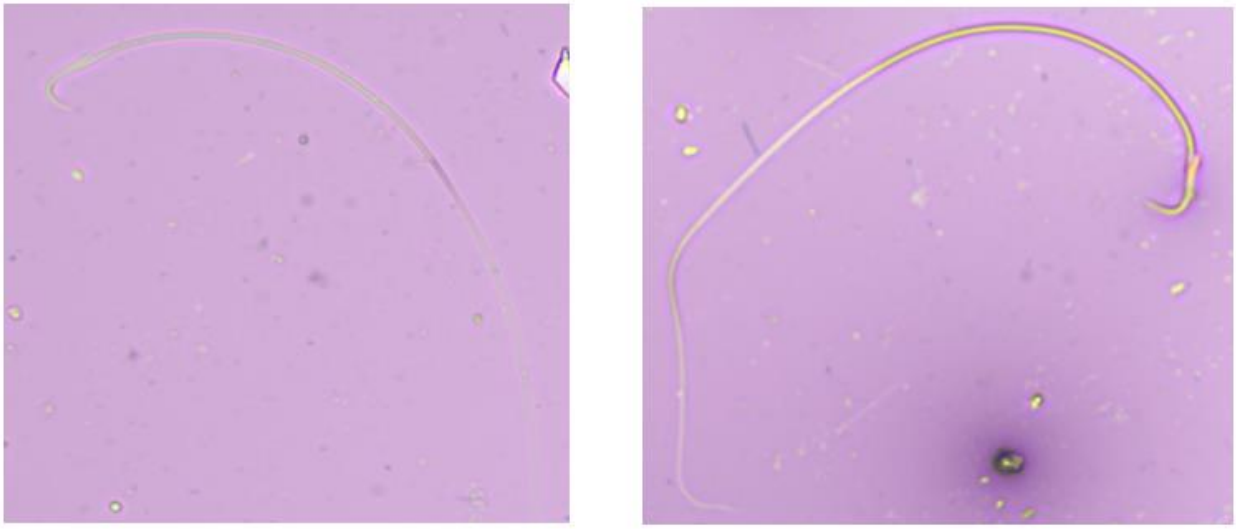


**Figure: 26. Microscopical image of Unstained (Viable) Sperm**

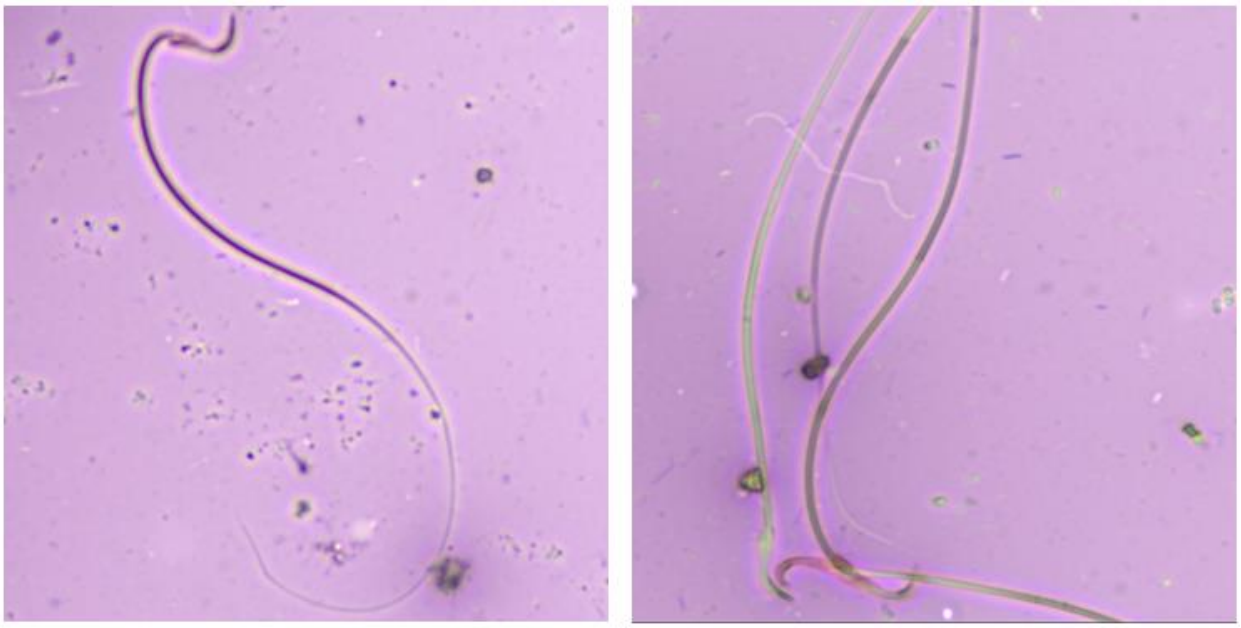


**Figure: 27. Microscopical image of Stained (Dead) Sperm**

### 7.6.4. Evaluation of Lipid Peroxidation in Testis

**Table no. 17. Effect on Lipid Peroxidation in Testis**

| S.No | Group | Lipid peroxidation (n moles of Malondialdehyde/g tissue) |
|---|---|---|
| 1 | Normal Control | 44.83 ± 1.14 |
| 2 | Disease Control | 118.50 ± 2.14 |
| 3 | Low Dose – *Naga parpam* | 71.83 ± 1.68 |
| 4 | High Dose – *Naga parpam* | 108.17 ± 0.95 |

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group, ** P<0.01 compared to disease control group**

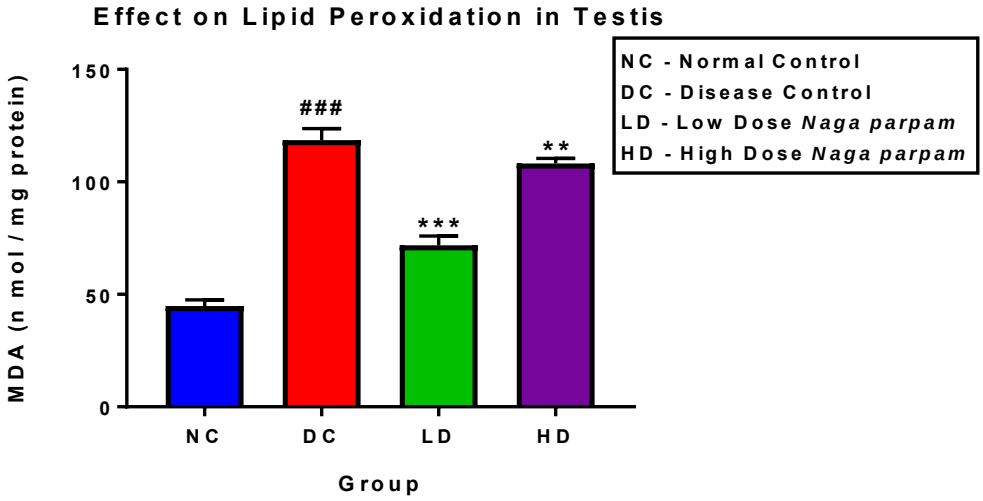



**Figure: 28. Effect of MCP and NP on lipid peroxidation in testis**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group, ** P<0.01 compared to disease control group**

The lipid peroxidation level was determined in rat testes and results are illustrated in **Table no.17 and Figure 28.** The MDA level in normal male rats was found to be **44.83 ± 1.14** n moles of Malondialdehyde/g tissue. The rats treated with Monocrotophos showed a highly significant (P<0.001) increase in the MDA level after 45 days treatment. The MDA level was

found to be **118.50 ± 2.14** n moles of Malondialdehyde/g tissue. In the rats which were treated with *Naga parpam* showed a significant reduction (P<0.001) in the MDA level. At the lower dose of 4 mg/kg, the MDA level was decreased to **71.83 ± 1.68** n moles of Malondialdehyde/g tissue. This MDA level however is still significantly (P<0.001) higher than the normal MDA level but significantly lower than MCP exposed rats. At the higher dose of 8 mg/kg, the *Naga parpam* was able to decrease the MDA level to **108.17 ± 0.95** n moles of Malondialdehyde/g tissue which is significantly lower (P<0.01) than the disease control value.

### 7.6.5. Evaluation of antioxidant enzymes

### A. SUPER OXIDE DISMUTASE LEVEL

**Table no. 18. Effect of *Naga parpam* and MCP on SOD level in Testis**

| S.No | Group | SOD ( IU/g tissue) |
|---|---|---|
| 1 | Normal Control | 27.50 ± 0.76 |
| 2 | Disease Control | 16.83 ± 1.19 |
| 3 | Low Dose – *Naga parpam* | 34.83 ± 1.01 |
| 4 | High Dose – *Naga parpam* | 29.00 ± 1.15 |

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

The SOD level was determined in rat testes and results are illustrated in **Table no.18 and Figure 29**. The SOD level in normal male rats was found to be **27.50 ± 0.76** IU/g tissue. The rats treated with Monocrotophos showed a highly significant reduction (P<0.001) in the SOD level after 45 days treatment. The SOD level was found to be **16.83 ± 1.19** IU/g tissue. In the rats which were treated with *Naga parpam* there was a significant increase (P<0.001) in the SOD level. At the lower dose of 4 mg/kg, the SOD level was increased to **34.83 ± 1.01** IU/g tissue. This SOD level is significantly higher (P<0.001) than the normal and MCP alone exposed SOD level. At the higher dose of 8 mg/kg, the *Naga parpam* was able to increase the SOD level to **29.00 ± 1.15** IU/g tissue and this SOD level was significantly higher (P<0.001) than the Disease control level but marginally higher than normal value

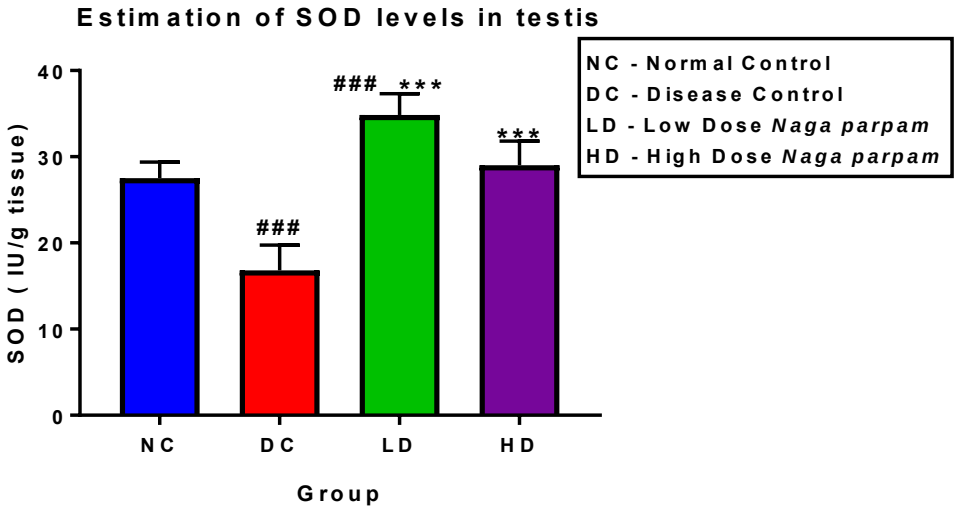



**Figure: 29. Effect of MCP and NP on SOD levels in testis**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

**B. CATALASE LEVEL**

**Table no. 19. Effect of *Naga parpam* and MCP in CAT level in male rat testes**

| S.No | Group | CAT (µ moles/g tissue/min) |
|---|---|---|
| 1 | Normal Control | 106.83 ± 0.83 |
| 2 | Disease Control | 48.17 ± 1.97 |
| 3 | Low Dose – *Naga parpam* | 78.38 ± 0.88 |
| 4 | High Dose – *Naga parpam* | 67.67 ± 0.76 |

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

The CAT level was determined in rat testes and results are illustrated in **Table no.19 and Figure 30**. The CAT level in normal male rats was found to be **106.83 ± 0.83** µ moles /g tissue/min. The rats treated with Monocrotophos showed a highly significant reduction (P<0.001) in the CAT level after 45 days treatment. The CAT level was found to be **48.17 ± 1.97** µ moles /g tissue/min. In the rats which were treated with *Naga parpam* showed a significant increase

(P<0.001) in the CAT level. At the lower dose of 4 mg/kg, the CAT level was increased to **78.38 ± 0.88** µ moles /g tissue/min. which are still significantly higher (P<0.001) than the MCP alone treated CAT level. At the higher dose of 8 mg/kg, the *Naga parpam* was able to increase the CAT level to **67.67 ± 0.76** µ moles /g tissue/min which is significantly higher (P<0.001) than MCP alone value.

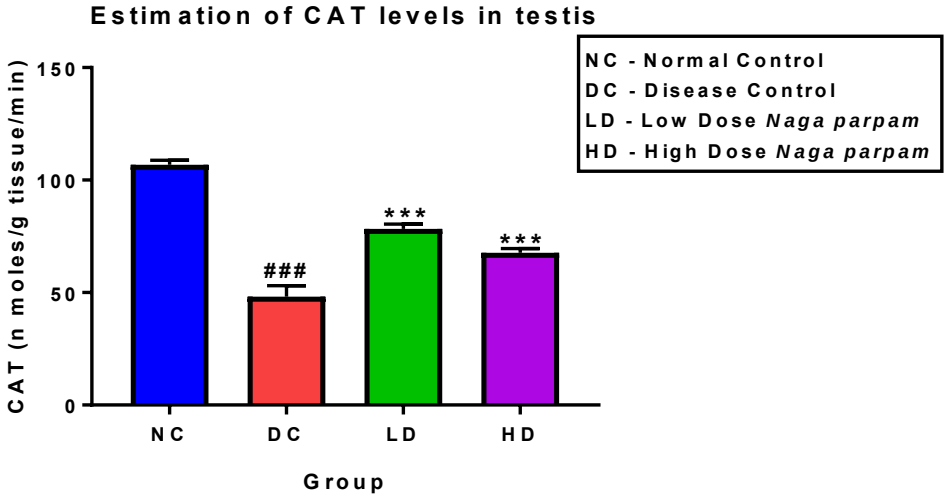



**Figure: 30. Effect of MCP and NP on CAT levels in testis**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

**C. GLUTATHIONE PEROXIDASE LEVEL**

**Table no. 20. Effect of *Naga parpam* and MCP in GPx level in male rat testes**

| S.No | Group | GPx (µ moles/g tissue/min) |
|---|---|---|
| 1 | Normal Control | 67.50 ± 0.76 |
| 2 | Disease Control | 30.17 ± 0.98 |
| 3 | Low Dose – *Naga parpam* | 55.50 ± 1.18 |
| 4 | High Dose – *Naga parpam* | 42.33 ± 1.05 |

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

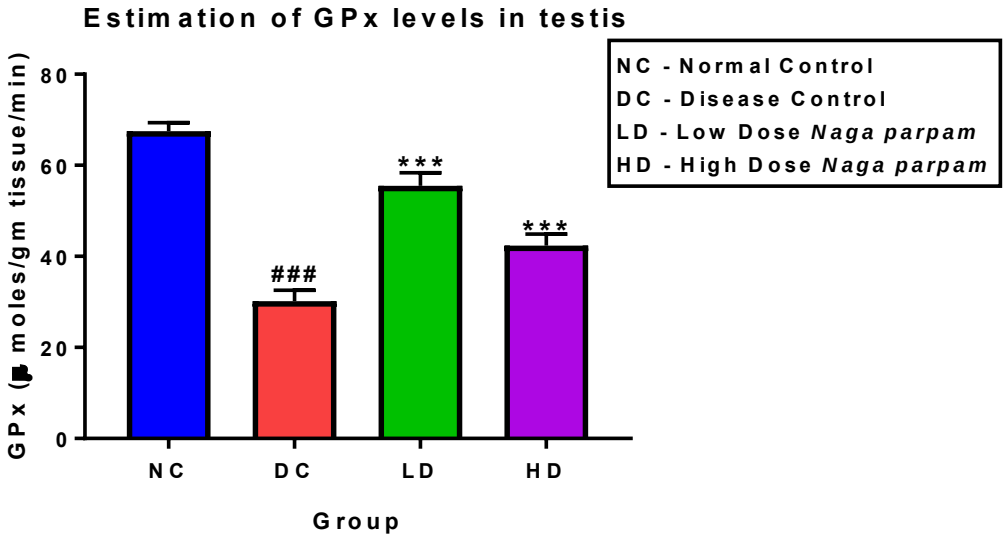



**Figure: 31. Effect of MCP and NP on GPx levels in testis**

**All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

The GPx level was determined in rat testes and results are illustrated in **Table no. 20 and Figure 31.** The GPx level in normal male rats was found to be **67.50 ± 0.76** µ moles /g tissue/min. The rats treated with Monocrotophos showed a highly significant reduction (P<0.001) in the GPx level after 45 days treatment. The GPx level was found to be **30.17 ± 0.98** µ moles /g tissue/min. In the rats which were treated with *Naga parpam* showed a significant increase (P<0.001) in the GPx level. At the lower dose of 4 mg/kg the GPx level was increased to **55.50 ± 1.18** µ moles /g tissue/min. This GPx level is still significantly lower (P<0.01) than the normal GPx level but significantly higher than MCP alone exposed rats. But at the higher dose of 8 mg/kg, the *Naga parpam* was able to increase the GPx level to **42.33 ± 1.05** µ moles /g tissue/min and this GPx level was significantly higher (P<0.001) than the MCP alone exposed value.

### 7.6.6. Estimation of serum testosterone level

**Table no. 21. Effect of *Naga parpam* and MCP in serum testosterone level in male rat testes**

| S.No | Group | Serum testosterone (ng/ml) |
|---|---|---|
| 1 | Normal Control | 2.43 ± 0.01 |
| 2 | Disease Control | 0.73 ± 0.002 |
| 3 | Low Dose – *Naga parpam* | 2.08 ± 0.05 |
| 4 | High Dose – *Naga parpam* | 1.16 ± 0.02 |

**All the values are expressed as Mean ± SEM (n=6). All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

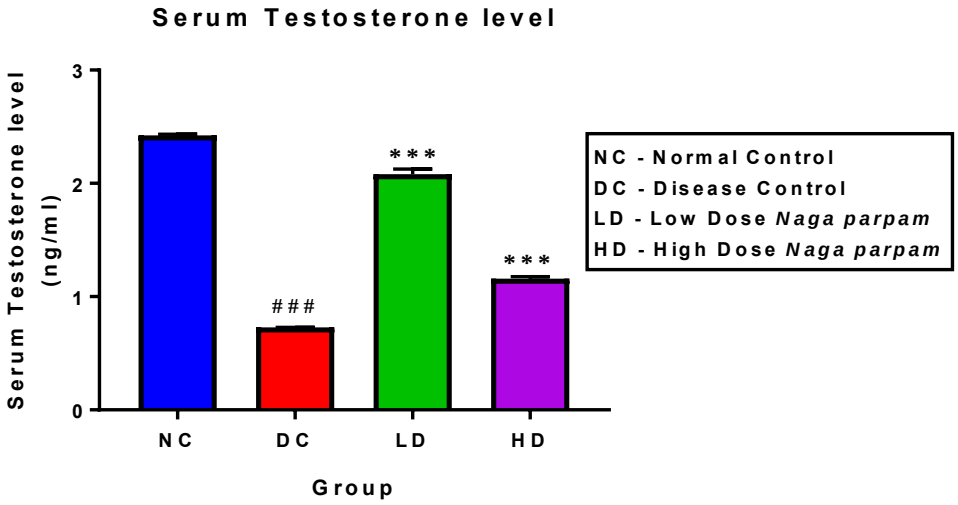



**Figure: 32. Effect of MCP and NP on serum testosterone level**

**All the values are expressed as Mean ± SEM (n=6). All the values are expressed as Mean ± SEM. (n=6), ###P<0.001 compared to normal control group, ***P<0.001 compared to disease control group**

The serum testosterone level was determined in rat testes and results are illustrated in **Table no. 21 and Figure 32.** The serum testosterone level in normal male rats was found to be **2.43 ± 0.01 ng/ml**. The rats treated with Monocrotophos showed a highly significant reduction (P<0.001) in the serum testosterone level after 45 days treatment. The serum testosterone level was found to be **0.73 ± 0.002 ng/ml**. In the rats which were treated with *Naga parpam* showed a significant increase (P<0.001) in the serum testosterone level. At the lower dose of 4 mg/kg the serum testosterone level was increased to **2.08 ± 0.05 ng/ml**. This serum testosterone level is still significantly lower (P<0.001) than the normal serum testosterone level but significantly higher than MCP alone exposed rats. But at the higher dose of 8 mg/kg, the *Naga parpam* was able to increase the serum testosterone level to **1.16 ± 0.02 ng/ml** and this serum testosterone level was significantly higher (P<0.001) than the MCP alone exposed value.

### 7.6.7. Histopathological studies

The testis section was examined for histological changes and the results were illustrated in the **Figure : 33 -36.**

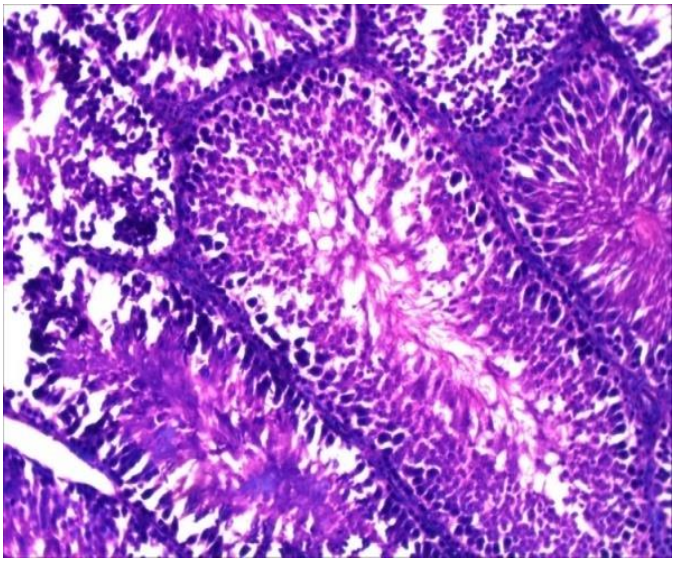


**Figure: 33. Photomicrograph of the rat testis - Normal control rats**

**Figure: 33.** The section of testis of normal control rats showed normal architecture of tubules with different types of germinal cells and lumen filled with spermatozoa

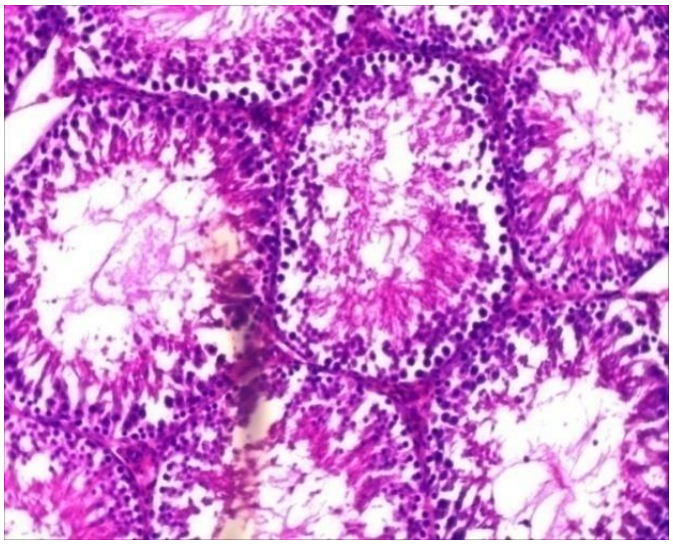


**Figure: 34. Photomicrograph of the rat testis - Disease control rats (MCP – 1.5 mg/kg)**

**Figure: 34:** showed detachment and loss of germinal cells and presence of vacuoles in the tubular lumen.

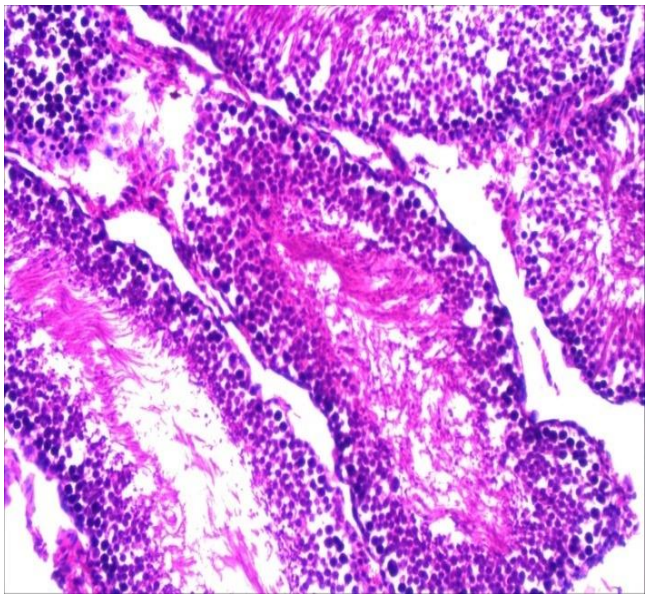


**Figure: 35. Photomicrograph of the rat testis -Low dose treatment (MCP + NP 4 mg/kg)**

**Figure: 35:** showing partial loss of germinal cells and decrease in number of vacuoles in the tubular lumen when compared with control rats

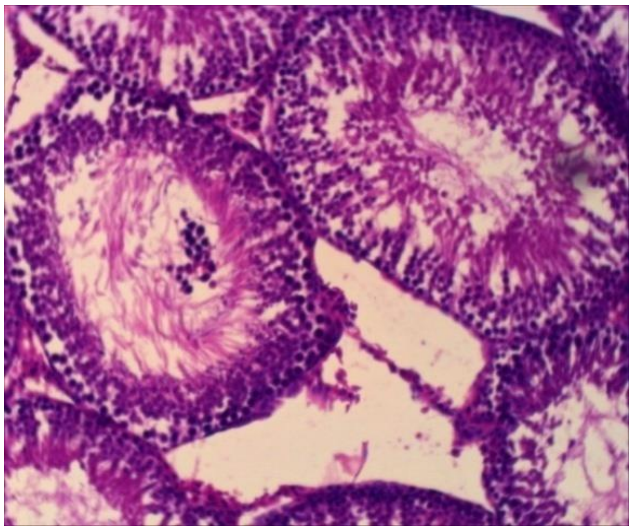


**Figure: 36 . Photomicrograph of the rat testis - High dose treatment (MCP + NP – 8 mg/kg)**

**Figure: 36:** showing recovery of germinal cells and luminal spermatozoa when compared with control rats, and shown almost same that of control rats histogram.

# DISCUSSION

# 8. DISCUSSION

## 8.1 CLASSICAL CHARACTERIZATION

*Naga parpam* complied with all the classical analysis methods. The formulation was found to be yellowish white in colour. Its taste was found to be tasteless i.e. absence of metallic taste. There were no shining particles in the parpam. It was observed that *Naga parpam* enters the furrows of finger and no fumes were found emerging out of it.

## 8.2 PHYSICOCHEMICAL ANALYSIS

In the physicochemical analysis, the pH of the drug is 8.05. It denotes that, it is alkaline in nature. So that, in the oral administration, the drug will get ionized in stomach and absorbed in intestine and sent directly to the portal system.

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredients of the drug. Thus low moisture content could get maximum stability and better shelf life. The loss on drying value of *Naga parpam* is found to be less than 1%, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature.

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug.

Total ash value indicates the amount of minerals and earthy materials present in the plant material. The total inorganic content (Zinc, Iron, Chloride, Silicate etc.,) present in the drug is measured through the Total ash and it is of 97.81% for *Naga parpam*.

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. It is 5.58% for *Naga parpam.*

## 8.3 PHYTOCHEMICAL ANALYSIS

Bioactive compounds are not present in *Naga Parpam.*

## 8.4 CHEMICAL ANALYSIS

From the chemical analysis, it is revealed that *Naga parpam* contains Zinc, Iron, Potassium, Chloride and Silicate.

## 8.5 *IN VITRO* ANTIOXIDANT ACTIVITY

From the results of in vitro antioxidant or free radical scavenging activity by DPPH free radical scavenging assay of standard (Ascorbic acid) and *Naga parpam* presented in **Table no.9 and Figure 10**. The $IC_{50}$ value of standard ascorbic acid **(23.82 µg/ml)** and *Naga parpam* (**83.73 µg/ml**) proved that they possess good antioxidant activity. The maximam percentage inhibition (75.42 ± 0.60 %) was found at a concentration of 320 µg/ml. The results obtained from $H_2O_2$ scavenging assay using standard (α - tocopherol) and *Naga parpam* were presented in **Table no.10 and Figure 13.** The maximam percentage inhibition (72.97 ± 0.26 %) was found at a concentration of 320 µg/ml and the $IC_{50}$ value of standard alpha tocopherol (**24.06 µg/ml**) and *Naga parpam* **(106.9 µg/ml)** proved that they possess good antioxidant activity.

## 8.6 *IN VIVO* STUDY

Male infertility is a serious health condition that affects a large number of men especially in developed countries. A large number of chemicals with endocrine disrupting potential are present in occupational and environmental settings, usually in the form of complex mixtures.

Insecticides are widely used to control the insects, but they are also harmful to humans. Insecticides are known to affect the reproductive system of both males and females. Organophosphates are one of the commonly used insecticides in India. Long term exposure to these cause reproductive damage in males and females.

Monocrotophos is one of the most commonly used organophosphate insecticides causing major toxic effects in the male reproductive system. Although there are several synthetic medicines available to treat reproductive damage, they are also associated with side effects. Hence there is an increase in the usage of traditional medicine especially in developing countries. *Naga parpam* which is a zinc based herbo-mineral siddha formulation have the potential to promote spermatogenesis. Hence this study was proposed to investigate the protective role of *Naga parpam* on Monocrotophos induced reproductive toxicity in adult male rats.

In the present study, adult male rats were administered Monocrotophos alone in one group and two other groups were given a combination of Monocrotophos and *Naga parpam* (at two dose levels). They were then assessed for changes in sperm parameters.

During the experiment, some signs of toxicity were noticed such as diarrhoea, mild tremor, huddling and food avoidance but no mortality occurred in all the exposed animals. The

variations in body and organ weight of control and treated groups were observed to determine the animals' physiological status.

The differences in body and sexual organ weights were taken as a valuable index for reproductive health and toxicological studies. To identify the toxic effects of Monocrotophos and the potential protective role of *Naga parpam* on rat reproductive system, sperm parameters, oxidative stress parameters, changes in the body and testicular weights were evaluated.

The present result revealed a decrease in body weight of Monocrotophos treated rats and this may be attributed to a decreased food intake as a result of diarrhoea and food avoidance observed following the administration of the insecticide. The changes in an organ's absolute or relative weight after administering a chemical or drug have been reported to indicate the substance's toxic effect. However *Naga parpam* partially reversed this body weight reduction.

The MCP-treated rats had lower prostate gland and epididymis weights. This finding could be elucidated by the low testosterone levels found in MCP-treated rats or by the epididymis's on-going androgenic stimulation, as well as the accessory sex organs's natural growth and activities. However *Naga parpam* given in combination with MCP, improves reproductive organ weight when compared to MCP alone exposed rats.

Pesticide's non-targeting effects are known as oxidative stress. Since oxidative stress can affect the steroidogenic potential of Leydig cells in the testis, as well as the germinal epithelium's ability to identify normal spermatozoa. It has been argued that oxidative stress is a significant factor in male reproductive failure. Under normal physiological conditions a delicate equilibrium occurs between the rate of creation of $H_2O_2$ via dismutation of $O_2$ by SOD activity and the rate of elimination of $H_2O_2$ by CAT.

As a result, any disturbance in this path will have an impact on the activity of the other enzymes in the chain. Obtained results revealed a significant reduction in the activity of testicular SOD (**16.83 ± 1.19** IU/g tissue) in all treated animals. Oxidative stress affects the tissue's ability to accommodate extra free radicals that can invade the thiol group of cysteine protein residues and polyunsaturated fatty acids of biological membranes.

However co-administration of *Naga parpam* along with MCP significantly increases the testicular SOD level when compared to MCP alone exposed rats. It is noteworthy to mention that

at low dose *Naga parpam* exposed rats , the SOD level (**34.83 ± 1.01** IU/g tissue) is significantly increased when compared to normal value (**27.50 ± 0.76** IU/g tissue) and high dose group showed marginally more than normal value (**29.00 ± 1.15** IU/g tissue).

MDA level, a stable polyunsaturated fatty acid (PUFA) breakdown product, is an important predictor of lipid peroxidation. A substantial elevation in the lipid peroxidation (MDA) levels has been obtained in testicular tissues after the MCP treatment in the current research. In rats which were simultaneously treated with *Naga parpam* and Monocrotophos, there was a significant reduction in the Malondialdehyde levels where low dose group shows more reduction than high dose group.

Androgens are the prominent male sex steroids and have the main function in the phenotype of male, sexual maturity, and maintenance the genital function and behaviour. In this study, significant reduction in testosterone concentration was observed after MCP exposure. The decline in testosterone level was attributed to the direct impact of pesticide on the testicular androgen biosynthesis pathway and its effect on the hypothalamus/frontal pituitary gland that may indirectly affect the testis and sexual function. Moreover, the low levels of the sex hormone effectively prevent the formation of sperms and the development of the seminiferous tubules and contribute to infertility. The reduction in the serum testosterone concentration may be owing to decreases in Leydig cell number and/or the damage of their structure caused by oxidative damage to testis tissues by pesticide. Both low dose treatment group and high dose treatment group shows significant increase in serum testosterone level when compared to MCP alone exposed group.

The sperm quality is a critical feature measurement for predicting sperm fertility and a sensitive index for studying the impact of various chemical and physical factors on reproductive cells. In the present study, MCP affect markedly sperm characteristics through a disruptive effect on sperm count and decrease the percentage of sperm motility, viability, and normality, which may cause infertility. However, co-administration of Monocrotophos and *Naga parpam* restored the reduced fertility parameters such as reproductive organ weights, especially testes, sperm counts, sperm motility and sperm viability as compared to Monocrotophos treated rats.

These harmful effects of the pesticides on sperm quality were related to the accumulation of pesticides in the testicular tissue, which affected the Sertoli cell group resulting in low sperm

formation and decreased sperm count. Another possible mechanism may be the inhibition of mitochondrial ATP output by uncoupling oxidative phosphorylation leading to ROS generation. This inhibition can affect the mitochondrial enzyme activities, suppressing the cell's ability to preserve their ATP levels and then damages the sperm microtubules' structure and disrupts their normal functions. In seminiferous tubules, testosterone is essential for the continual development of distinct germ cell generation. Thus, the decrease in testosterone levels found in this study may contribute to the sloughing of germ cells from the seminiferous epithelium as well as the reduction in sperm characteristics. Moreover, the decrease in sperm motility may be due to a change in fructose composition or erosion of the sperm's microtubules. Interestingly, a reduction in sperm viability supports the decline in motility. This decrease could be related to pesticide's negative impact on the epididymis of exposed rats. Sperm morphology is a powerful predictor of the human body and therefore, testicular health that responds strongly to physical, physiological, and environmental stresses, much more than any other organ. An aberration in spermatogenesis is the leading cause of primary sperm abnormality.

Pesticide impacts in various animal tissues and organs can be detected quickly using histological alterations. The current study demonstrated that testicular damage is consistent with prior research that found varying degrees of testicular dysfunction, testosterone deficiency, spermatogenesis suppression and oxidative stress in rats exposed to insecticide. Histological abnormalities can be directly attributed to the pesticide-induced cytotoxic impact by causing oxidative stress on the seminiferous tubule, which led to the production of the free oxygen radicals, antioxidants changes, LPO, and/or indirectly via a decrease in testosterone levels. Therefore, the low level of testosterone observed in this study may have deleterious effects on the seminal tube and may lead to germ cell detachment from the seminal epithelium leading to germ cell apoptosis and finally, reproductive toxicity.

These testicular damages were significantly mitigated by co-administration of *Naga parpam*, indicating that *Naga parpam* protects the testicular tissue from the histological damage mediated by Monocrotophos. The protective role of Naga parpam against Monocrotophos induced male reproductive toxicity may be due to presence of zinc which has very significant role in maintaining normal male reproductive function.

It is noteworthy to mention that even though both low dose (4 mg/kg) and high dose (8 mg/kg) of *Naga parpam* mitigate the male reproductive toxicity induced by Monocrotophos, it was observed that low dose – *Naga param* ameliorates more effectively than the high dose exposed group which in agreement with Azam Javadi *et al* (2020) who demonostrated a time- and dose-dependent cytotoxic effect for ZnO nanoparticles in SSCs and testicular cells which suggested that more caution should be taken when using these materials on a daily basis. The short time exposure to a low concentration of ZnO NPs could promote spermatogenesis without impairing Sertoli cells in their niche.[80] This demands further research for dose optimisation because of increased potency of *Naga parpam* due to its nano particle size. The unique properties of nanomaterials, including increased surface area, greater cellular uptake, and antioxidative properties, provide nanomaterials with new or enhanced physical and chemical reactions

# SUMMARY AND CONCLUSION

## SUMMARY AND CONCLUSION

*Naga Parpam* has been chosen as the drug to perform the research on its anti-infertility action based on the literature review. *Naga parpam* which was prepared by repeated calcination of Zinc metal in presence of *Eclipta prostrata* juice leads to detoxification of metal and size reduction to nanoparticles.

Zinc plays a very important role in human body. Zinc has only single oxidation state i.e. $Zn^{2+}$ and it is found in more than 300 enzymes, present in all six classes of enzymes characterized by the International Union of Biochemistry and required for their catalytic activity. Zn is an important trace mineral that people need to stay healthy. This element is second only to iron in its concentration in the body. Zn is found in cells throughout the body. Zn is involved in numerous aspects of cellular metabolism. Further, Zn is essential for genetic stability and function. This indicates that if Zn is substituted by normal means, which is not in the nano scale, there will be further accumulation in the blood plasma, and it cannot penetrate the cell. If Zn has to be supplied to the cells, which are deficient in Zn, Zn has to be given in a form, which is non-toxic and also it has to penetrate the cells. Hence, Zn should be substituted as nano particle, so that it penetrates the cell. Hence, *Naga Parpam* is considered for this study.

This study concluded that the preliminary classical organoleptic analysis of *Naga parpam* indicated that it complied with all the classical properties of parapm (i.e lustreless, tasteless, odourless etc).

This study concluded that the preliminary physicochemical analysis of *Naga parpam* indicated that it was alkaline in nature and had high percentage of total ash value (i.e presence of high inorganic content)

The phytochemical analysis of *Naga parpam* indicated the absence of bioactive organic compounds and the chemical analysis revealed that it contains zinc which was thought to be responsible for its protecting role against MCP induced fertility changes.

*In vitro* Antioxidant activity of *Naga parpam* was evaluated by DPPH free radical scavenging assay and Hydrogen peroxide radical scavenging assay. Results showed that *Naga parpam* had good anti-oxidant potential.

The *in-vivo* study was conducted with four groups of male Wistar albino rats (six in each) to assess the protective role of monocrotophos induced fertility changes. Group I – Normal control, Group II – Disease control, Group III – Low dose treatment group and Group IV – High dose treatment group.

All animals were induced with monocrotophos except control group of rats for 45 days daily by oral route. Test groups were treated with low and high dose respectively once a daily for 45 days. Various parameters were evaluated such as body weight, reproductive organ weight, sperm parameters (i.e sperm count, viability, motility), testicular lipid peroxidation, antioxidant enzymes level in testis, serum testosterone level and histopathological examination. The results were analysed using one-way ANOVA and p value were calculated to find the significance of the results.

Results has shown that treatment with *Naga parpam* has been effective in ameliorating the detrimental effects of Monocrotophos such as reduced body weight, reproductive organ weight, sperm count, viability, motility, testicular lipid peroxidation, testicular antioxidant enzymes and histopathological changes. Even though both low dose (4 mg/kg) and high dose (8 mg/kg) of *Naga parpam* mitigate the male reproductive toxicity induced by Monocrotophos, it was observed that low dose – *Naga param* ameliorates more effectively than the high dose exposed group. This demands further research for dose optimisation because of increased potency of *Naga parpam* due to its nano particle size. The unique properties of nanomaterials, including increased surface area, greater cellular uptake, and antioxidative properties, provide nanomaterials with new or enhanced physical and chemical reactions

From this study it is concluded that the *Naga parpam* have the ability to reverse the infertility induced by Monocrotophos in male rats. There was an improvement in sperm parameters and the antioxidant levels in animals which were treated with the *Naga parpam* along with the insecticide Monocrotophos. Since Reactive oxygen species play an important role in causing male reproductive changes and *Naga parpam* which contains zinc a well-known antioxidant, it is inferred that the activity of *Naga parpam* could be related to its antioxidant properties.

Further studies are needed to be carried out for determining exact mechanism of action of *Naga parpam* for treating monocrotophos induced infertility.

# REFERENCES

## 10. REFERENCES


1. Maric T *et al*. Environmental and occupational exposures associated with male infertility. Arh Hig Rada Toksikol. 2021;72:101–113.
2. World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018.
3. Agarwal A, Mulgund A, Hamada A, Chyatte M R. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:37.
4. Shadnia et al. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. Hum. Exp. Toxicol. 2005;24:439–445.
5. Ashiru O.A, Odusanya O.O. Fertility and occupational hazards: review of the literature. Afr. J. Reprod. Health . 2009;13:159–165.
6. Abdollahi *et al*. Pesticides and oxidative stress: a review. Med. Sci. Monit. 2004;10:141–147.
7. Sifakis S, M Parmpas M, Soldin O P, Tsatsakis A *et al*. Pesticide exposure and health related issues in male and female reproductive system. Pesticides—Formulations, Effects, Fate. 2011;25:495–526.
8. The WHO Recommended Classification of Pesticides By Hazard And Guidelines To Classification : 2004.
9. Heong K L *et al*. Research Methods in Toxicology And Insecticide Resistance Monitoring of Rice Planthoppers. International Rice Research Institute. 2011;2:19-34.
10. Amajad Iqbal Kazi , Anna Oommen. The Effect of Acute Severe Monocrotophos Poisoning on Inhibition, Expression and Activity of Acetylcholinesterase in Different Rat Brain Regions. Neurotoxicology. 2012;33:1284–1290.
11. Joshi S.C. And Bansal B. Reproductive Toxicity of Monocrotophos in Male Rats. International Journal of Toxicology And Applied Pharmacology 2012; 2(1):6-11.
12. Velmurugan G, Venkatesh Babu D, Subbiah Ramasamy. Prolonged Monocrotophos Intake Induces Cardiac Oxidative Stress And Myocardial Damage In Rats, Toxicology. 2013;307:103– 108.
13. Preeti R. Revankar, S.K. Shyama. Genotoxic effects of monocrotophos, an organophosphorous pesticide, on an estuarine bivalve, Meretrix ovum. Food And Chemical Toxicology. 2009;47:1618–1623

14. Vibhuti Mishra *et al*. Evaluation of Genotoxicity of Monocrotophos and Quinalphos in Rats and Protective Effects of Melatonin. Integr Pharm Toxicol Genotoxicol. 2015;1(1): 33-42.
15. Amajad Iqbal Kazi , Anna Oommen *et al*. Monocrotophos Induced Oxidative Damage Associates With Severe Acetylcholinesterase Inhibition In Rat Brain. Neurotoxicology. 2012;33:156–161
16. Radhika P. Rao, Basappa B. Kaliwal *et al*. Monocrotophos Induced Dysfunction on Estrous Cycle and Follicular Development In Mice. Industrial Health. 2002; 40:237–244.
17. Raheem A A, Ralph D *et al*. Male infertility: causes and investigations. Trends Urol Men's Health. 2011;2(5):8-11.
18. Jungwirth A *et al.* European Association of Urology working group on male infertility. Eur Urol. 2012;62(2):324–332.
19. Cocuzza M, Alvarenga C, Pagani R *et al*. The epidemiology and etiology of azoospermia. Clinics. 2013;68(S1):15–26.
20. Hamada A J, Esteves S C, Agarwal A. A comprehensive review of genetics and genetic testing in azoospermia. Clinics. 2013;68(S1):39–60.
21. Baker K, Sabanegh J E.Obstructive azoospermia: reconstructive techniques and results. Clinics. 2013;68(S1):61–73.
22. Wosnitzer M, Goldstein M, Hardy MP. Review of azoospermia. Spermatogenesis. 2014;4:e28218.
23. Kumar R. Medical management of non-obstructive azoospermia. Clinics. 2013;68(S1):75–79.
24. Kupis Ł, Dobroński PA, Radziszewski P. Varicocele as a source of male infertility: current treatment techniques. Cent European J Urol. 2015;68:365–370.
25. Fode M, Krogh-Jespersen S, Brackett NL, Ohl DA, Lynne CM, Sønksen J. Male sexual dysfunction and infertility associated with neurological disorders. Asian J Androl. 2012;14(1):61–68.
26. Pizent A, Blanka T, Tanja Z. Reproductive toxicity of metals in men. Arh Hig Rada Toksikol.2012;63(1):35–46.
27. Jung A, Schuppe HC. Influence of genital heat stress on semen quality in humans. Andrologia. 2006;39(6):203–215.

28. Kolesnikova L I, Kolesnikov S I, Kurashova N A, Bairova T A. Causes and factors of male infertility. Vestn Ross Akad Med Nauk. 2015;5:579–584.
29. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. Fertil Steril. 2001;75(2):237–248.
30. Harris ID, Aaron KS, John CP. Ultrasonographer experience does not impact outcomes following ultrasound-guided embryo transfer. Fertil Steril. 2009;92(3):918–922.
31. Harris ID, Fronczak C, Roth L, Meacham RB. Fertility and the aging male. Rev Urol. 2011;13(4):184–190.
32. Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB, LuconAM. Sperm concentration and normal sperm morphology decrease and follicle-stimulating hormone level increases with age. BJU Int. 2005;96(7):1087–1091.
33. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the massachusetts male aging study. J Urol. 1994;151(1):54–61.
34. Momoh AR, Idonije BO, Nwoke EO, Osifo UC, Okhai O, Omoroguiwa A, Momoh AA. Pathogenic bacteria-a probable cause of primary infertility among couples in Ekpoma. J Microbio Biotech Res. 2017;1(3):66–71.
35. Moretti E, Capitani S, Figura N, Pammolli A *et.al*. The presence of bacteria species in semen and sperm quality. J Assist Reprod Genet. 2009;26(1):47–56.
36. Junior W B, Shiratsu R, Pinto V. Approach in sexually transmitted diseases. An Bras Dermatol. 2009;84(2):151–159.
37. Gimenes F, Souza R P, Bento J C, Teixeira J J *et al.* Male infertility: a public health issue caused by sexually transmitted pathogens. Nat Rev Urol. 2014;11:672–687.
38. Triunfo S, Lanzone A. Impact of maternal under nutrition on obstetric outcomes. J Endocrinol Investig. 2015;38(1):31–38.
39. Wong W Y *et al*. Male factor subfertility: possible causes and the impact of nutritional factors. Fertil Steril. 2000;73(3):435–442.
40. Nargund VH. Effects of psychological stress on male fertility. Nat Rev Urol. 2015;12:373–382.
41. Sharma R, Biedenharn K R, Fedor J M, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol. 2013;11:66.

42. Brydøy M, Fosså S D, Dahl O, Bjøro T. Gonadal dysfunction and fertility problems in cancer survivors. Acta Oncol. 2007;46(4):480–489.
43. Abdel-Raheem A, Ralph D, Minhas S. Male infertility [review article]. Br J Med Surg Urol. 2012;5:254–268.
44. Jelnes J E, Knudsen L E. Stainless steel welding and semen quality. Reprod Toxicol. 1988;2(3–4):213–215.
45. Tchounwou P B, Yedjou C G, Sutton D J. Heavy metal toxicity and the environment. Mol Clin Environ Toxicol. 2012;101:133–164.
46. Tielemans E, Van Kooij R, Te Velde E R, Burdorf A *et al*. Pesticide exposure and decreased fertilization rates in vitro. Lancet. 1999;354(9177):484–485.
47. Kaur R P, Gupta V, Christopher A F, Bansal P *et al*. Potential pathways of pesticide action on erectile function – a contributory factor in male infertility. Asian Pacific Journal of Reproduction. 2015;4(4):322–330.
48. Kanter M, Aktas C, Erboga M *et al*. Heat stress decreases testicular germ cell proliferation and increases apoptosis in short term: an immune histochemical and ultrastructural study. Toxicol Ind Health. 2013;29:99–113.
49. Durairajanayagam D, Agarwal A, Ong C *et al*. Causes, effects and molecular mechanisms of testicular heat stress. Reprod. Biomed. 2015;30(1):14–27.
50. Sheynkin Y, Jung M, Yoo P, Schulsinger D *et al*. Increase in scrotal temperature in laptop computer users. Hum. Reprod. 2005;20(2):452–455.
51. Jorgensen N, Andersen A G, Eustache F, Irvine D S *et al*. Regional differences in semen quality in Europe. Hum Reprod. 2001;16(5):1012–1019.
52. Schilling K, Toth B, Rösner S, Strowitzki T *et al*. Prevalence of behaviour-related fertility disorders in a clinical sample:results of a pilot study. Arch Gynecol Obstet. 2012;286(5):1307–1314.
53. Platz E A, Leitzmann M F, Rimm E B *et al*. Alcohol intake, drinking patterns, and risk of prostate cancer in a large prospective cohort study. Am J Epidemiol. 2004;159(5):444–453.
54. Jensen TK, Bonde JP, Joffe M. The influence of occupational exposure on male reproductive function. Occup Med (Lond). 2006;56(8):544–553.

55. Choudhary R, Chawala V K, Soni N D, Jayant K *et al.* Oxidative stress and role of antioxidants in male infertility. Pak J Physiol. 2010;6(2):54–59.
56. Walczak-Jedrzejowska R, Jan K W, Jolanta S. The role of oxidative stress and antioxidants in male fertility. Central European Journal of Urology. 2012;66(1):60–65
57. Jungwirth A, Giwercman A, Tournaye H, Diemer T *et al*. European Association of Urology working group on male infertility. Eur Urol. 2012;62(2):324–332.
58. Practice Committee of American Society for Reproductive Medicine (ASRM). Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril. 2012;98(2):294–301.
59. Patil P S, Shi R, Ashalata D P, Anita R G e*t al*. Immature germ cells in semen – correlation with total sperm count and sperm motility. J Cytol. 2013;30(3):185–189.
60. Vasan S S. Semen analysis and sperm function tests: how much to test? Indian J Urol. 2011;27(1):41–48.
61. Kumar R. Medical management of non-obstructive azoospermia. Clinics. 2013;68(S1):75–79.
62. Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. Singap Med J. 2009;50(4):336–347.
63. Madbouly K, Al-Hooti Q, Albkri A, Ragheb S *et al*. Clinical, endocrinological and histopathological patterns of infertile Saudi men subjected to testicular biopsy: a retrospective study from a single center. Urol Ann. 2012;4(3):171–199.
64. Abdullah L, Bondagji N. Histopathological patterns of testicular biopsy in male infertility: a retrospective study from a tertiary care center in the western part of Saudi Arabia. Urol Ann. 2011;3(1):19–23.
65. Mythili Ramalingam, Suresh Kini, Ahir Mahmood. Review of Male fertility and infertility. Obstet, Gynaecol Reprod Med. 2014
66. Iyad Khourdaji, Haerin Lee, Ryan P. Smith. Frontiers in hormone therapy for male infertility. Transl Androl Urol. 2018;7(3):353-66.
67. Ali A. Dabaja, Peter N. Schlegel. Review of Medical treatment of male infertility. Transl Androl Urol. 2014;3(1):9-16.
68. Dama Madhukar and Singh Rajender. Review of Hormonal Treatment of Male Infertility: Promises and Pitfalls. Journal of Andrology. 2009;30(2):95-112.

69. Peter Y.Liu and David J.Handelsman. The present and future state of hormonal treatment for male infertility. Hum Reprod Update. 2003;9(1):9-23.
70. Ahmad Majzoub A, Ashok Agarwal. Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol. 2018;16(1):113–124.
71. Gvozdjakova A, Kucharska J, Dubravicky J, Mojto V *et al*. Coenzyme Q10, α-tocopherol and oxidative stress could be important metabolic biomarkers of male infertility. Dis Markers 2015. DOI:10.1155/2015/827941.
72. Balakrishnan I, Sharief Sulta D, Krishnamurthy V K, Raman R *et al*. Effect of *Naga Parpam*, a Zinc-Based Siddha Medicine, on Hyperlipidemia in Rats. Journal of Complementary and Integrative Medicine. 2009;6:1-2.
73. Arun Sudha, V.S.Murty, T.S.Chandra. Standardization of Metal-Based Herbal Medicines. American Journal of Infectious Diseases. 2009;5(3):193-199.
74. Willi Paul, Chandra P. Sharma. Characterizations of *Rasa Chenduram* (Mercury Based) and *Naga Parpam* (Zinc Based) Preparations: Physicochemical and *In vitro* Blood Compatibility Studies. Trends in Biomaterials and Artificial Organs. 2012; 25(4):124-132
75. Viswanathan et al. The hormesis of *Naga parpam* in experimentally induced pyelonephritic mice. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(1): 644-648
76. R. Sahulhameed *et al.* Physicochemical Characterization and Instrumental Analysis of the Siddha Mineral Drug Naga Parpam. International Journal of Medicine and Pharmaceutical Research.2015;3(1):897–901
77. Ramanathan R & Ramasamy R. Characterization of zinc oxide based nano siddha medicine naga parpam – potential nutritive supplement in cancer. Journal of Pharmaceutical and Scientific Innovation. 2019;8(3):100-104
78. Rajamaheswari *et al*. SEM Characterization and Antiproliferative effect of novel siddha nano formulation *Nagarasa parpam* on hep2 cell line. International Journal of Modern Pharmaceutical Research. 2019;3(3):08-10
79. Hai-Tao Gao *et al*. Zinc supplement ameliorates phthalates-induced reproductive toxicity in male rats. Chemosphere. 2020;246:1-9.

80. Azam Javadi *et al*. Short time exposure to low concentration of zinc oxide nanoparticles upregulates self-renewal and spermatogenesis-related gene expression. International Journal of Biochemistry and Cell Biology. 2020;127:105822.
81. Viswanathan Vinotha *et al*. Synthesis of ZnO nanoparticles using insulin-rich leaf extract: Anti-diabetic, antibiofilm and anti-oxidant properties. Journal of Photochemistry & Photobiology, B: Biology. 2019;197: 111541.
82. Siba Soren *et al*. Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method. Microbial Pathogenesis. 2018; 119 :145–151.
83. S. Rajeshkumar *et al*. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme and Microbial Technology. 2018;117:91-95.
84. Amna Sirelkhatim *et al*. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Lett. 2015; 7(3):219–242.
85. Badkoobeh *et al*. Effect of nano-zinc oxide on doxorubicin- induced oxidative stress and sperm disorders in adult male Wistar rats. Iranian Journal of Reproductive Medicine. 2013;11(5):355-364.
86. Vismaya, P.S. Rajini. Oral Exposure to the Organophosphorus Insecticide, Monocrotophos Induces Intestinal Dysfunction In Rats. Food And Chemical Toxicology. 2014;71:236–243.
87. Vibhuti Mishra, Sapneh Sharma, Shilpa Khatri, Nalini Srivastava *et al*. Evaluation of Genotoxicity of Monocrotophos And Quinalphos In Rats And Protective Effects of Melatonin. Integr Pharm Toxicol Genotoxicol. 2015;1(1):33-42.
88. Xiaona Zhang, Lei Gao, Kunfeng Yang, Hua Tian *et al*. Monocrotophos Pesticide Modulates The Expression of Sexual Differentiation Genes And Causes Phenotypic Feminization In Zebrafish (Danio Rerio). Comparative Biochemistry And Physiology. 2013;157: 33–40.
89. Velmurugan G, Venkatesh Babu D.D, Subbiah Ramasamy. Prolonged Monocrotophos Intake Induces Cardiac Oxidative Stress And Myocardial Damage In Rats. Toxicology. 2013;307:103– 108.

90. Amajad Iqbal Kazi, Anna Oommen. Monocrotophos Induced Oxidative Damage Associates With Severe Acetylcholinesterase Inhibition In Rat Brain. Neurotoxicology. 2012;33:156–161.
91. Apurva Kumar R. Joshi, P.S. Rajini. Hyperglycemic And Stressogenic Effects of Monocrotophos In Rats**:** Evidence For The Involvement of Acetylcholinesterase Inhibition. Experimental And Toxicologic Pathology. 2012;64:115– 120.
92. Santosh K. Yaduvanshi, Anupama Ojha, Satish C. Pant *et al*. Monocrotophos Induced Lipid Peroxidation And Oxidative DNA Damage In Rat Tissues. Pesticide Biochemistry And Physiology. 2010;97:214–222.
93. Kalyan Chakravarthi B. Rambabu Naravaneni, Phili G. H. *et al*. Investigation of Monocrotophos Toxic Effects On Human Lymphocytes At Cytogenetic Level. African Journal of Biotechnology. 2009;8(10):2042-2046.
94. Radhika P. Rao, Basappa B. Kaliwal. Monocrotophos Induced Dysfunction on Estrous Cycle And Follicular Development In Mice. Industrial Health. 2002;40:237–244.
95. Anonymous, Formulary of Siddha Medicine. The Indian Medical Practitioners' Co-operative Pharmacy and Stores Ltd., Madras 1972:12.
96. Dr.R.Thiyagarajan, L.I.M, Gunapadam Thaathu Jeevam Vaguppu, part II and III, 4th edition 2004.
97. Avani Pareek, Nitu Bhatnagar. Physico-chemical characterization of traditionally prepared Yashada bhasma. Journal of Ayurveda and Integrative Medicine. 2020;11(3):228-235.
98. The Ayurvedic Pharmacopoeia of India, Part – I, Volume – IX, Government of India, Ministry of AYUSH, 2016- First Edition.
99. Shimada K., Fujikawa K., Yahara K., Nakamura T *et al*. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J. Agr. Food Chem. 1992; 40:945–948.
100. Perumal, P. and Saravanabhavan, K. Antidiabetic and antioxidant activities of ethanolic extract of *Piper betle* L. leaves in catfish, *Clarias gariepinus*. *Asian J Pharm Clin Res*. 2018;11(3):194-198.

101. R.J. Ruch, S.J. Cheng, J.E. Klaunig. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea Carcinogenesis, 1989;(10):1003.

102. Keser, Serhat, et al. Hydrogen peroxide radical scavenging and total antioxidant activity of hawthorn. Chem J 2.1 (2012): 9-12.

103. Toxicity studies of *Naga sangu parpam*, *Nanju noolum maruthuva neethi noolum*, National Institute of Siddha,2010.

104. Ilango B, Dawood sharief S, Vinothkumar K, Rajkumar R., Prathiba D and Sukumar E, Histopathological studies of the effect of Naga parpam, a zinc based drug of siddha medicine, in rats, Journal of Cell and Tissue Research. 2009;9(2):1869-1873.

105. Kanchana Gang U, Hemalatha C, Kishori. B, Protective Role of Date Fruit Extract Against Chlorpyrifos-Induced Reproductive Toxicity In Albino Male Rats. International Journal of Green Pharmacy. 2018;12 (1):113-119.

# ANNEXURE

**MADRAS MEDICAL COLLEGE, CHENNAI – 600003**

**INSTITUTIONAL ANIMAL ETHICS COMMITTEE**

**PROCEEDINGS**

**PRESENT: Dr. A. JERAD SURESH, M.Pharm., Ph.D., MBA**

**Roc. No: 5/AEL/IAEC/MMC/2022 Dated: 01-11-2021**

Sub: IAEC, MMC, Ch-3 – Approval of Laboratory Animals – Regarding

Ref: IAEC Meeting held on 21-10-2021

This order is issued based on the approval by the Institutional Animal Ethics Committee Meeting held on 21-10-2021, Thursday.

| | |
|---|---|
| Project Proposal ID Number | 03/2021-2022 |
| CPCSEA Registration Number | 1917/GO/ReBi/2016/CPCSEA<br>Valid till 19-9-2026 |
| Name of the Researcher with ID Number | H. BOOBESH<br>261926053 |
| Name of the Guide | Dr. R. Indumathy, M.Pharm., Ph.D., |
| Project Title | Protective role of ***Naga parpam*** – a zinc based herbo-mineral formulation against Monocrotophos induced fertility changes in Male Wistar Rats. |
| Date of submission of proposal to IAEC | 07-10-2021 |
| Date of IAEC meeting | 21-10-2021 |
| Date of submission of modified proposal to IAEC | 22-10-2021 |
| Date of Approval | 21-10-2021 |
| Validity of the Approved Proposal | One Year |
| Number & Species of Laboratory Animals Approved | **24 Male Wistar Rats Approved** |

10/2/22

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai-600003

PRINCIPAL
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

To
Dr. R. Indumathy, M.Pharm., Ph.D.,
Assistant Professor, Dept. of Pharmacology,
College of Pharmacy,
MMC, Ch-3.

Copy to:
Special Veterinary Officer, Animal Experimental Laboratory,
Madras Medical College, Ch-3.

# MADRAS MEDICAL COLLEGE

## MULTIDISCIPLINARY RESEARCH UNIT

(A Unit of Department of Health Research, MoHFW, Govt. of India)

# GOOD CLINICAL PRACTICE WORKSHOP – RECENT UPDATE 2021

## Certificate Of Attendance

*This is to certify that Dr/Mr/Miss/Mrs.* Boobesh. H

*participated in one day GCP workshop conducted at Madras Medical College organized by Multidisciplinary Research Unit, Chennai on 30th Tuesday, March 2021.*

*This educational activity has been reviewed and awarded* ***10 credit points under category - II*** *by the Centre of Accreditation, The Tamil Nadu Dr. MGR Medical University.*

**Dr. K. NARAYANASAMY,**
Nodal Officer, MRU, MMC

**Dr. K. BASKARAN,**
Organizing Secretary & Vice Principal, MMC

**Dr. E. THERANIRAJAN,**
DEAN, MMC & RGGGH

# INSTITUTE OF PHARMACOLOGY

## MADURAI MEDICAL COLLEGE, MADURAI

# CERTIFICATE

This is to certify that ~~Dr~~/~~Mrs~~/Mr. H. BOOBESH ..... has participated as a ~~Speaker~~/ Delegate in the VIRTUAL CME ON "GOOD CLINICAL PRACTICE IN HUMAN RESEARCH" conducted by The Institute of Pharmacology, Madurai Medical College, Madurai on Friday, 06.11.2020

Dr. J.Sangumani MD.,D.Diab.,
DEAN
Government Rajaji Hospital &
Madurai Medical College.,
Madurai

Dr. Malar Sivaraman MD.,
ORGANIZING SECRETARY,
Director & Head of the
Department,
Government Rajaji Hospital &
Madurai Medical College,
Madurai.

# 71st Indian Pharmaceutical Congress

Theme: Healthcare System - Role of Regulators

*Chennai, TamilNadu.*

Theme: Pharma vision 2030

## Certificate of Skill Development

*This is to certify that Mr. / Ms.* BOOBESH.H

*of* MADRAS MEDICAL COLLEGE

*has participated in the Animal Handling Skill Development of 71st Indian Pharmaceutical Congress 2019 held at Sri Ramachandra Institute of Higher Education and Research (DU) during 20th to 22nd December 2019, Chennai, TamilNadu.*

| *Shri. Ravi Uday Bhaskar* | *Dr. P.V. Vijayaraghavan* | *Dr. M. Dhilip Kumar* | *Dr. S. Thanikachalam* |
|---|---|---|---|
| *President - 71st Ipc 2019* | *Chairman - Loc* | *Secretary - Loc* | *Convener - Skill Development* |

Organised by
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Hosted by
All India Drugs Control Officers' Confederation

# 71st Indian Pharmaceutical Congress

## Theme: Healthcare System - Role of Regulators

Theme Pharma vision 2030

# Certificate

*This is to certify that ~~Prof./Dr./Mr./Ms.~~* BOOBESH. H
*of* COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE *has participated in the 71st Indian Pharmaceutical Congress held at Sri Ramachandra Institute of Higher Education and Research (DU), from 20th to 22nd December 2019, Chennai, Tamil Nadu.*

| **Shri. Ravi Uday Bhaskar** | **Dr. P.V.Vijayaraghavan** | **Dr. M. Dhilip Kumar** | **Dr. T.V. Narayana** |
|---|---|---|---|
| President - 71st IPC 2019 | Chairman - LOC | Secretary - LOC | General Secretary - IPCA |

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