Effect of Methanolic Extract of *Mucuna Pruriens* on Hormonal Modulation and Sperm Parameters in Male Rat

Chitra Kalyanaraman & PD Gupta

ABSTRACT

Objective: Studies on hormonal changes is an effective method for assessing infertility conditions. A widely used pesticide 1, 2 Dibromo 3 Chloropropane (DBCP) causes infertility by damaging testis in humans. This in turn disturbs Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), sex steroid hormone levels. The seed extract of Mucuna pruriens (M. pruriens) is well known antioxidant and is expected to improve the sperm quality after exposure to DBCP. Method: Male Sprague-Dawley rats were divided into four Groups. Group I – Control received 0.9% saline, Group II – single dose of 50mg/kg 1, 2-Dibromo 3 Chloro Propane (DBCP) dissolved in Dimethyl sulphoxide (DMSO) (1ml) was administered for 30 days, Group III – Methanolic extract of Mucuna pruriens (200mg/kg body weight) was administered for 45 days after 50mg/kg of 1,2 dibromo 3 chloropropane (DBCP) treatment (30 days). Group IV – 200mg/kg/day BW methanolic extract of M. pruriens for 45 days. Results: The body, testis, and epididymis weights of all the rats were taken. DBCP induced group II toxicity bearing animals were significantly decreased when compared to group I control animals. In group II DBCP-induced toxicity rats, there was a reduction in sperm morphology, concentration, and motility as well as follicle stimulating hormone (FSH), testosterone, and luteinizing hormone (LH) levels.

Keywords: methanolic seed extract, sperm motility, sperm count, luteinizing hormone, follicle stimulating hormone, testosterone.

Classification: UDC: 613.95

Language: English

London Journals Press

LJP Copyright ID: 925642
Print ISSN: 2631-8490
Online ISSN: 2631-8504

London Journal of Research in Science: Natural and Formal

Volume 23 | Issue 5 | Compilation 1.0

Effect of Methanolic Extract of *Mucuna Pruriens* on Hormonal Modulation and Sperm Parameters in Male Rats

Chitra Kalyanaraman[α] & PD Gupta[σ]

ABSTRACT

Objective: Studies on hormonal changes is an effective method for assessing infertility conditions. A widely used pesticide 1, 2 Dibromo 3 Chloropropane (DBCP) causes infertility by damaging testis in humans. This in turn disturbs Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), sex steroid hormone levels. The seed extract of Mucuna pruriens (M. pruriens) is well known antioxidant and is expected to improve the sperm quality after exposure to DBCP. Method: Male Sprague-Dawley rats were divided into four Groups. Group I – Control received 0.9% saline, Group II – single dose of 50mg/kg 1, 2-Dibromo 3 Chloro Propane (DBCP) dissolved in Dimethyl sulphoxide (DMSO) (1ml) was administered for 30 days, Group III – Methanolic extract of Mucuna pruriens (200mg/kg body weight) was administered for 45 days after 50mg/kg of 1,2 dibromo 3 chloropropane (DBCP) treatment (30 days). Group IV – 200mg/kg/day BW methanolic extract of M. pruriens for 45 days. Results: The body, testis, and epididymis weights of all the rats were taken. DBCP induced group II toxicity bearing animals were significantly decreased when compared to group I control animals. In group II DBCP-induced toxicity rats, there was a reduction in sperm morphology, concentration, and motility as well as follicle stimulating hormone (FSH), testosterone, and luteinizing hormone (LH) levels. DBCP-induced group II toxicity-bearing animals' above-mentioned parameters improved after treatment with Mucuna pruriens seed extract. Conclusion: The administration of M. pruriens seed extract improves sperm quality and Hormone levels in the DBCP exposed group III rats.

Keywords: methanolic seed extract, sperm motility, sperm count, luteinizing hormone, follicle stimulating hormone, testosterone.

Author α: Dr. ALM Post Graduate Institute of Basic Medical Sciences, Taramani, Chennai, TamilNadu, India.
σ: Former Director grade Scientists, Centre for Cellular and Molecular biology, Hyderabad, India.

I. INTRODUCTION

Infertility is an International problem that involves young couples having unprotected intercourse. According to global evidence, infertility in male ranges from 20-70% (1). Declining male reproductive health is a major concern among the population of reproductive age. Various environmental factors causing male infertility are described in the scientific and medical literature (2). Numerous external and internal factors can increase the production of reactive oxygen species (ROS) above and beyond the capacity of cellular antioxidants, leading to oxidative stress. Exposure to Endocrine - Disrupting Chemicals (EDCs) is ubiquitous in our everyday lives and may result in oxidative stress, which can have an impact on human reproduction and development (3). Pesticides such as Pyrethroids, Organophosphates, Peroxyacetic acids, Carbamates and Organochlorines have been investigated in the study of male fertility (4). Mankind that promoted a negative effect on the environment due to the increase of its own requirements and technology producing unfavorable consequences on the surroundings.

1, 2 Dibromo 3 chloropropane (DBCP), is one such organochlorine pesticide that was used widely for the control of Agricultural and Domestic pests. Twenty years after it was banned, it is still found in the environment, because it takes 140 years to degrade completely (5). Pesticides' effects on sperm parameters have been related in numerous studies (6). Teitelbaum (1999) established that DBCP causes a significant reduction in spermatogenesis among pesticide manufacturing workers. Also, hormones play role in the fertility and infertility conditions. Thus, investigations on hormonal changes may be useful tool in the assessment of both fertility and infertility conditions. Whorton *et al.*, 1977, Kelce *et al.*, 1995, Bernard *et al.*, 2007 (8-10) also verified that DBCP also have estrogenic effects in males by blocking androgen receptors. It is well established that the acute stress that produces excess cortisol decreases the Testosterone production and suppress male sex hormones such as Luteinizing hormone (LH), Follicular-Stimulating Hormone (FSH) (11-13).

Mucuna pruriens (*M. pruriens*) belongs to the family Fabaceae, native to tropical countries from Africa and Asia, including India, Bangladesh, Srilanka & China (14). The seeds have been considered as magic velvet bean in several published reviews (15,16). It has a long history in Indian Ayurvedic medicine, where it is used to treat for Diarrhea, Sexual Debility, Tuberculosis, Impotence, Rheumatic disorders (17,18). Suresh *et al.*, (2009) (19) reported that *M. pruriens* helps in increasing the semen quality and it acts as aphrodisiac. *M. pruriens* seed is economically available all year and it contains phytochemicals such as alkaloids, glycosides, saponins (20). Due to its richness in various biological activities, it has been characterized by in vitro antioxidant activity, anti-microbial agents, and natural antioxidants (21). *M. pruriens* is not only a reproductive enhancer, but also an important natural material for the treatment of male infertility (22). Thus, there is a great possibility that this plant may act through the mechanism of free radical removal in the management of reproductive toxicity.

II. MATERIALS AND METHODS

2.1 Animals

Healthy adult male Sprague- Dawley rats (8 weeks old), weighing between 160-220g were used in the present study. All rats were kept in plastic cages under the experiment room condition at Laboratory Animal Unit PGIBMS, University of Madras, India. The rats were housed under conditions of controlled temperature (26±2°C) with 12 h light and 12 h dark exposure. The rats received a standard rat pellet diet and water *ad libitum*.

2.2 Animal Ethics

The 24 Sprague- Dawley Male Rats were obtained from the Central Animal House facility, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai, Tamilnadu, India. Rats were used as per the guidelines from the Institutional Animal Ethics Committee (07/021/08).

2.3 Experimental design

The rats were divided into four groups of six animals each in this experiment. Group I - Animals were treated as control (0.9% saline). Group II - To induce reproductive dysfunction, a single dose of 50mg/kg body weight of 1,2 Dibromo 3 chloropropane (DBCP) dissolved in Dimethyl sulphoxide (DMSO) (1ml) was administered intragastrical for 30 days. Group III - After 1,2 dibromo 3 chloropropane (DBCP) treatment, a methanolic extract of *M. pruriens* (200mg/kg/day body weight) was administered intragastrical for 45 days. For 45 days, Group IV received a 200mg/kg/day body weight methanolic extract of *M. pruriens*. Dosage of *M. pruriens* was selected according to Suresh *et al.* (2009) (19) with ±200 mg to confirm effective concentration.

2.4 Collection of samples

At the end of the experiment, the animals were anesthetized by mild ether and euthanized by cervical dislocation. Blood was collected and was centrifuged at 4 ℃, 13,000 rpm for 15 min to separate the serum from the blood cells. After that, the testosterone and cortisol hormone levels were analyzed from the blood serum. The abdominal region was wiped with normal saline and the scrotum was dissected out. The testes, epididymis plus vas deferens were rapidly collected and their fat pads surrounding tissues were removed before weighed and recorded. Then the right testis and right epididymis plus vas deferens were fixed in Bouin's fixative (85 ml of saturated picric acid added to 10 ml of 40% formaldehyde and made up to 100 ml with glacial acetic acid) for histological examinations.

2.5 Preparation of Methanolic M. Pruriens Seed Extract

The seeds of *Mucuna pruriens* were purchased from a local country drug shop, Chennai Tamilnadu, India. The seeds of *M. pruriens* were shade dried and then coarsely powdered. A known weight of the seed powder was soaked in 100% methanol and kept at room temperature (22 ± *°C*) for 96 h. Then it was filtered, and the process was repeated three times. The extract was concentrated to obtain a semisolid viscous brown mass, which is “crude extract” by using a water bath. This crude extract is then resuspended in water and injected to rats for the experimental studies.

III. SPERM ANALYSIS

3.1 Sperm morphology

Sperm morphology was evaluated by determining the percentage of normal and abnormal forms by Diff-Quick staining method (23). To assess the percentage of morphologically abnormal sperm, the cauda of epididymis was rinsed with 0.5 mL of physiological saline (0.9% NaCl) to obtain a sperm suspension. Aliquots of sperm suspension were stained with 2% eosin. Hundred spermatozoa per animal were analyzed microscopically at 400× magnification and counted spermatozoa with abnormal traits as follows: twisted body, detached head, round tails, and abnormal neck.

3.2 Epididymal Sperm Count

Epididymal sperm count of the control and treated animals was determined by the method as described by Latchoumycandane and Mathur, (2002) (24). An incision was made through the cauda of epididymis, light pressure was applied to this region, and sperm was extruded. 5µl aliquot of epididymal sperm was diluted with 95 µl diluent (5% sodium bicarbonate, 10ml 0.35 % formalin and 0.25 g trypan blue) and approximately 10 µl of this diluted sperm was allowed to stand for 5 min in a humit chamber to prevent drying. The sedimented sperms were counted under the light microscope at 400x magnifications. The measured sperm number was multiplied by the dilution factor to yield the total sperm count.

3.3 Sperm concentration and motility

Epididymal sperms were counted with a hemocytometer using a method described by Yokoi *et al.*, (2003) (25). 5 µl of sperm suspension was diluted with 95 µl of phosphate-buffered saline (PBS, pH 7.4) solution. Approximately 10 µl of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and was allowed to stand for 5 minutes. The settled sperms were counted with the help of a light microscope at 400x magnifications. Under light microscope, the sperm were counted within two upper and lower counting chambers in triplicate examinations and calculated to be sperm concentration (million cells/mL).

The percentage of motile sperm was evaluated microscopically in each sample by viewing a drop of sperm suspension obtained from left cauda epididymis diluted with Tris buffer solution (3.63 g of Tris-hydroxymethyl aminomethane, 0.50 g of glucose, 1.99 g of citric acid and 100 ml of distilled water) on a prewarmed (37°C) slide and cover slip and observed under light microscopy at 400x magnification. Motility estimations were performed from four different fields in each sample. The mean of the four estimations were used as the final motility score (Sonmez *et al.,* 2005) (26).

3.4 Dead and abnormal sperms

20 µl of sperm suspension was mixed with an equal volume of 0.05% eosin-Y (Sigma Chemicals). After 2 min of incubation at room temperature, slides were viewed under microscope at 400x magnification. Dead sperms appeared pink and live sperms were not stained. Two hundred sperms were counted for each sample and viability percentage was calculated. For the analysis of morphological abnormalities, sperm smears were drawn on clean slides, and allowed to dry in air overnight. The slides were stained with 1% eosin-Y and 15% nigrosin. This was examined at 400x magnifications for morphological abnormalities such as amorphous, head less, bicephalic, coiled or abnormal tails (Wyrobek *et al.,* 1983) (27).

3.5 Sperm Vitality tests

3.5.1 Hypo-osmotic swelling test (HOS)

The Hypo Osmotic Swelling (HOS) test was performed as described by Jeyendran *et al.,* (1984) (28). This is based on the semi permeability of the intact cell membrane, which causes spermatozoa to swell under hypo-osmotic conditions when an influx of water results in an expansion of cell volume. Through HOS test the ability of the plasma membrane to transport water by subjecting the spermatozoa to hypo-osmotic conditions can be measured.

3.5.2 Dye exclusion tests (Eosin and Nigrosin stain) Sperm vitality test

Eosin-nigrosin staining was used to assess the vitality of sperms. This test was studied according to the method of WHO (1999) (23). One drop of sperm suspension was mixed with two drops of 1 % eosin Y. After 30 seconds, three drops of 10 % nigrosin were added and mixed well. Thin smears were then prepared and observed under the light microscope at 400X magnification. Viable sperms remained colorless while non-viable sperms-stained red.

3.6 Hormonal Assays

Blood samples were separated by centrifugation at 10000 rpm for 15 minutes to determine the testosterone (T), follicular stimulating hormone (FSH), and luteinizing hormones (LH) levels (29). Serum FSH was assayed by solid phase tube method by making use of a commercial kit obtained from Diagnostic Products Corporation (DPC), USA. Serum LH was assayed by solid phase coated tube methodology by making use of a commercial kit obtained from Diagnostic Products Corporation (DPC), USA. Serum testosterone was assayed by solid phase coated tube methodology by making use of a commercial kit obtained from Diagnostic Products Corporation (DPC), USA.

3.7 Statistical analysis

Data were presented as Mean ± Standard Deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups by using SPSS.7.5 students version.

IV. RESULTS

4.1 Bodyweight and Organ weight

In this present investigation, the administration of DBCP has caused significant reduction in the weight of testis and accessory sex organs in group II with respect to its controls. On *M. pruriens* supplementation the weight of testis and accessory sex organs recovered remarkably ($p \leq 0.05$), from the DBCP toxicity (Table 1). The body weight of group III male rats shows significant improvement after treated with *M. pruriens* seeds.

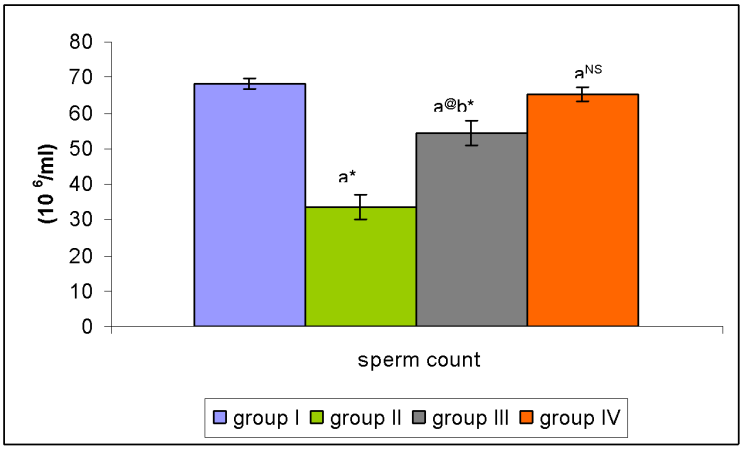
Table 1: Body and Organ weight of Experimental animals

Parameters	Group I (Control)	Group II (DBCP)	Group III (DBCP + *M. pruriens*)	Group IV (*M. pruriens*)
Body weight(g)	160.33±4.33	132.30±4.43a*	146.06± 2.51a* b*	157.49±3.19a^{NS}
Testicular weight (g)	2.67±0.16	1.61±0.09a*	2.38±0.16a* b*	2.69±0.10 a ^{NS}
Testis weight relative (g)	1.76± 0.04	1.14 ±0.09 a*	1.63± 0.11 a$^{@}$ b*	1.79 ±0.05 a ^{NS}
Epididymis weight (g)	0.379± 0.01	0.28 ±0.02 a*	0.320±0.02 a$^{\#}$ b*	0.359±0.01 a ^{NS}

Values are expressed as mean ± SD for six animals in each group, a – Group II, III, IV compared with Group I, b – Group III compared with Group II. The significance level of $p<0.05$.

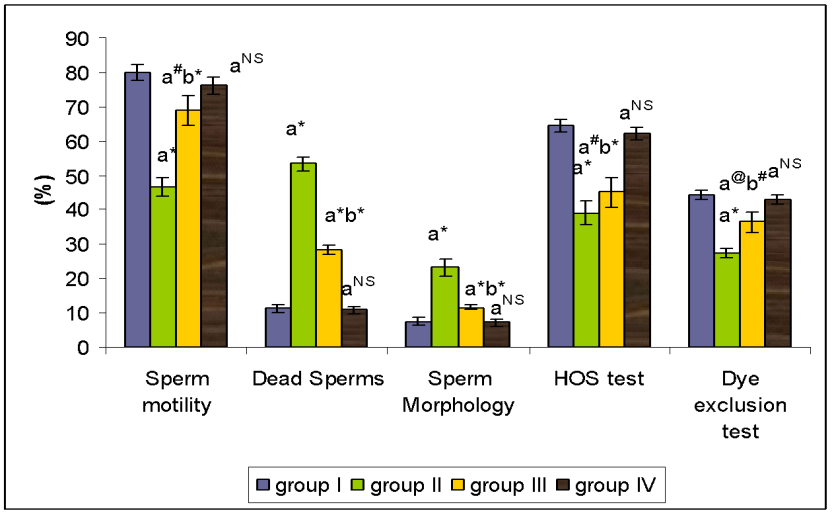
4.2 Sperm Count

The results demonstrated the decrease in sperm count of rats under stress. Fig 1 shows the sperm count of experimental animals, and it is inferred that the sperm count was drastically reduced in group II DBCP treated animals when compared to group I control animals. Conversely upon treatment with *M. pruriens*, the sperm count was significantly increased in group III *M. pruriens* treated animals and were comparable to that of group II toxicity bearing animals.



Each value represents mean ± SD, a – Group II, III, IV compared with Group I, b – Group III compared with Group II $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

Fig 1: Sperm Count in Experimental animal

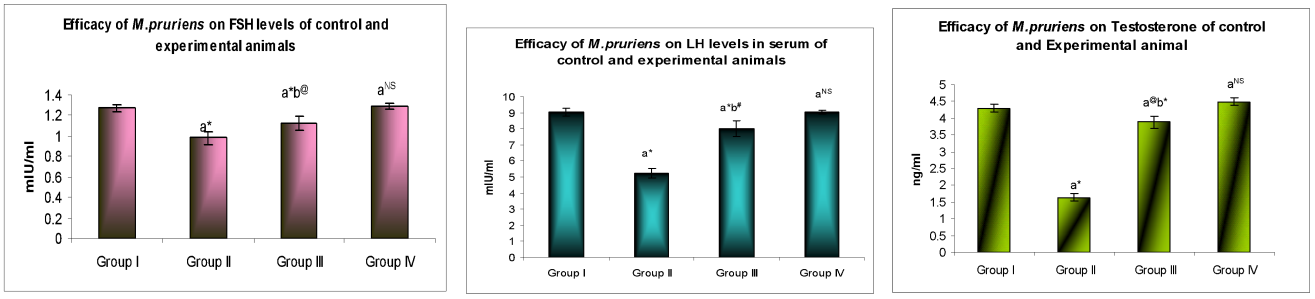


Each value represents mean ± SD, a – Group II, III, IV compared with Group I, b – Group III compared with Group II $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

Fig 2: Sperm motility, Morphology and Vitality of Control and Experimental animals

Fig 2 demonstrates Sperm motility, morphology, viability by HOS and Dye exclusion test. The negative impact of DBCP on spermatozoa was confirmed by an increased abnormality in the sperm head. Also, abnormal spermatozoa with twisted bodies, detached heads, abnormal necks, and round tails were predominantly noticed. On DBCP exposure, the proportion of sperm with progressive movement was significantly ($p<0.05$) decreased. Whereas the treatment with methanolic extract of *M. pruriens*, the percentage of abnormal sperms significantly decreased ($p<0.05$), and movement was significantly improved. In contrast to this, the sperm Motility, Viability and Dye exclusion test shows Decrease level in group II DBCP exposed rats (Fig 2) and was significantly reverted to near normal in *M. pruriens* extract treated group III animals. Not much changes observed in group IV *M. pruriens* alone treated animals when compared to group I control animals.

4.3 Hormonal Parameters



Each value represents mean ± SD, a – Group II, III, IV compared with Group I, b – Group III compared with Group II $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

Fig 3a, 3b, 3c: FSH, LH & Testosterone level of Control and Experimental animals

Testosterone, luteinizing hormone and follicle stimulating hormone levels in the male experimental rats were observed (Fig. 3a,3b &3c). The level of these hormones in the experimental rats followed similar trend. They were significantly less in group II rats which received 50mg/kg of DBCP. Contrarily, upon administration of *M. pruriens* extract these hormone levels were significantly increased in group III comparable to that of group II toxicity bearing rats. There was however no significant difference ($p > 0.05$) in the level of these hormones recorded in group 4 rats compared to control group.

V. DISCUSSION

In recent years, the major health concern is toxic effects of drug and environmental chemicals on Human reproductive system. Reproductive toxicity can be defined as adverse effect of chemical substance on sexual function and fertility effects on male and females or dysfunction of the reproductive system. The oxidative stress that causes dysfunction of male reproductive hormone, which could eventually lead to male infertility. According to global evidence, infertility is a common and severe health problem affecting 20-70% of male population (1). Most of those patients were most likely exposed to toxicants, which may have contributed to their infertility (30).

In the present investigation, DBCP treated rats showed a significant decrease in body weight and testicular weight when compared to the control. This may be due to the disturbance in the general metabolic functions of the rats exposed to toxicant. It is reported that in toxicity conditions, the body weight of Experimental Animal is significantly reduced (31). In addition to these, hormonal changes may also be one of the reasons for weight loss (32). Also, the loss of testicular and epididymal weight may be due to reduced bioavailability of sex hormones (33). Additionally, it is suggested that the decrease in body weight may be for increased degeneration of lipids and proteins because of the direct effects of reproductive toxicant (34). Treatment with *M. pruriens* in Group III animal shows significant increase in the body and testicular weight. This may be due to the cytoprotective property of the seed extract (31).

The sperm count in the epididymis is one of the most sensitive tests for evaluating spermatogenesis (35). In the current study, administration of DBCP to group II animals caused the epididymal epithelium to degenerate, which resulted in a significant decrease in sperm count compared to group I control animals. Upon treatment with *M. pruriens* increase the sperm count due to the huge amounts of phenolic constituents that are characterized by free radical scavenging and high antioxidant activities which suppress the free radical mediated disturbances in sperm. This is in well accordance with Suresh *et al.*, (2009) and Chitra (2022) (19,22) revealed an increase in sperm count and motility by the seed extracts of *M. pruriens*.

Sperm motility is often used as a marker of chemically induced testicular toxicity. Sperm movement is important for sperm functional capacity and the assessment of sperm movement is useful for detection or evaluation of male reproductive toxicity (36). It is reported that the increase in the Oxidative stress leads to a decreased sperm motility, damage to the acrosome membranes and inability of the sperm to fertilize (37). In the present study, DBCP leads to diverse cells and oxidative damage to sperms can lead to DNA damage, alter membrane functions, impair motility.

Decrease in sperm motility and abnormal morphology of sperm was noticed in DBCP treated rats when compared to control rats. On the contrary, treatment with seed extract of *M. pruriens*, significantly increased the sperm motility and in contrast reduced the abnormal sperm morphology. This may be due to the protective and restorative effect rendered by the methanolic seed extract of *M. pruriens*. In view of this, Shami *et al.*, (2009) (38) have also reported that antioxidants play a major role in improving sperm morphology and sperm count.

The hypo-osmotic swelling (HOS) test was used for evaluating the functional integrity of human spermatozoa membranes, by evaluating its reaction under hypoosmotic conditions. In the present investigation exposure to DBCP caused severe plasma membrane damage in the sperm due to the generation of free radicals. The administration of *M. pruriens* stabilized and restored the normal membrane potential due to the presence of glycoside, saponins and sterols present in the seed extract. In this view Yousef *et al.*, (2005) (39) also reported that the active compounds such as glycosides and

Lafuente *et al.*, (2000) (40) reported that Leutinizing hormone (LH), Follicle stimulating hormone (FSH) and Testosterone are required for normal spermatogenesis. Elevated levels of FSH and LH are as deleterious as sub-normal levels (41). Previous studies have shown that decreases in serum testosterone, testicular LH receptors, and Leydig cell damage might be adversely affected by the toxicant (42-44). Testosterone is the main male gonadal hormone produced by the interstitial cells of the Leydig cells in the testis. The role of testosterone and gonadotrophin has been studied extensively in androgen- deficient rats using different models and in prevention of degeneration of spermatogenic cells (45). A reduction in testosterone level could be the primary cause of induction of infertility induced by the compound. Testosterone is one of the major indexes of androgenicity (46,35). In the present study, there is a decline in testosterone levels in the DBCP treated group II rats. The reduced serum testosterone levels support the possibility of reproductive tract alterations due to androgen deficiency.

The morphological changes of the seminiferous tubule including Leydig and Sertoli cells would indicate damage to the reproductive system. In the present study the administration of DBCP decreases the FSH and LH levels, due to the toxic action of DBCP. This is in consistence with the finding of Pease *et al.*, (1991) (47) that chemical toxicant causes persistent dysfunction of Leydig cells which disturb normal testosterone levels. In this connection, Fiorini *et al.*, 2004 (48) have also reported that LH concentration was rise and Testosterone levels were decreased during the complete failure of Leydig cells. However, hormonal production may be reduced in rats due to the seminiferous tubular damage (49). On the contrary , treatment with *M. pruriens in* group III significantly decreases the toxic effects of DBCP and the level of FSH, LH and testosterone were increased. This might be due to the presence of the active constituents such as flavonoids and saponins which directly or indirectly scavenge the oxidative damage to different cells and organs while normalizing their function. In this regard, Doshi *et al.,* (2003) (50) have reported that flavonoids and antioxidants remarkably reduce oxidative damages in the cells. Based on the results of this study, future research could investigate male rat sterility using a fertility test. In addition, sire litters can be examined after mating.

VI. CONCLUSION

In conclusion, the current study supports the methanolic extract of *M. pruriens* has a remarkable fertility effect in male rats. These results conclude that the seed extract of *M. pruriens* significantly increase sperm quality by elevating antioxidant enzyme activity and improve Hormone levels. Because of its efficacy, it can be considered as potential seed extract for further pharmaceutical development in the treatment of infertility.

ACKNOWLEDGEMENT

I would like to express deep and Sincere gratitude to my Research Supervisor Late Dr. M.P. Balasubramanian, Professor, Dr. ALM PGIBMS, Taramani, Chennai-113. for giving me the opportunity to do research and invaluable guidance throughout my Research work. My Sincere Thanks goes to Dr. K. Pushkala, SDNB Vaishnav college for women for the continuous support and encouragement throughout the paperwork.

REFERENCES

1. Agarwal A, Parekh N, Panner Selvam M.K. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. World J Mens Health, 37(3): 296–312 (2019).
2. Nykolaichuk P. Roksolana, Oleksandr S, Fedoruk, Volodymyr V. Vizniuk. Impact of Environmental Factors on Male Reproductive Health Wiad Lek. 73(5):1011-1015 (2020).
3. Irma Virant-Klun, Senka Imamovic-Kumalic and Bojana Pinter. Endocrine-Disrupting Chemicals (Bisphenols, Phthalates, and Parabens) with Human Semen Quality *Antioxidants*, *11*(8): 1617 (2022).
4. Perry MJ. Effects of environmental and occupational pesticide exposure on human sperm: a systematic review. Hum Reprod ,14: 233-42 (2008).
5. Reed J, Kinzel V, Cheng HC, Walsh DA. Circular dichroic investigations of secondary structure in synthetic peptide inhibitors of cAMP-dependent protein kinase: a model for inhibitory potential. *Biochemistry*, 1;26(24) :7641-7 (1987).
6. Krzastek Sarah C, Jack Farhi, Marisa Gray and Ryan P. Smith. Impact of environmental toxin exposure on male fertility potential**.** Transl Androl Urol., 9(6): 2797–2813 (2020).
7. Teitelbaum DT. The toxicology of 1,2-dibromo-3- chloropropane (DBCP): a brief review. Int J Occup Environ Health, 5:122-6 (1999).
8. Whorton D, Krauss R.M, Marshall S, Milby T.H. and Stubbs. Function in DBCP exposed pesticide workers. *Lancet,* ii, 161-167 (1977).
9. Kelce WR, Stone CR, Laws SC. Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature; 375:581-5 (1995).
10. Bernard L, Martinat N, Lécureuil C. Dichlorodiphenyltrichloroethane impairs follicle stimulating hormone receptor-mediated signaling in rat Sertoli cells. Reprod Toxicol; 23:158-64 (2007).
11. Herman JP. Neural control of chronic stress adaptation. Front Behav Neurosci. 7:61 (2013).
12. LinH, YuanK, ZhouH-y ,BuT, SuH, LiuS, ZhuQ, WangY, HuY, ShanY. Time-course changes of steroidogenic gene expression and steroidogenesis of rat Leydig cells after acute immobilization stress. Int J Mol Sci. 15(11): 21028–21044 (2014).
13. Mohamadpour M, Noorafshan A, Karbalay S, Talaei T, Aliabadi E. Protective effects of curcumin co-treatment in rats with establishing chronic variable stress on testis and reproductive hormones. Int J Reprod Biomed. 15(7):447–452 (2017).
14. Fung SY, Tan NH, Sim SM, Marinello E, Guerranti R, Aguiyi JC. *Mucuna pruriens* Linn. seed extract pretreatment protects against cardiorespiratory and neuromuscular depressant effects of *Naja sputatrix* (Javan spitting cobra) venom in rats. Indian Journal of Experimental Biology. 49(4):254-259 (2011).
15. Lampariello LR, Cortelazzo A, Guerranti R, Sticozzi C, Valacchi G. The magic velvet bean of *Mucuna pruriens*. Journal of traditional and complementary medicine. 2(4):331-339 (2012).
16. Kavitha, Thangamani C. Amazing bean *Mucuna pruriens*: A comprehensive review. Journal of Medicinal Plants Research. 8:138-143 (2014).
17. Suryawanshi, Prajakta P Kamble, Vishwas A Bapat, Jyoti P Jadhav. Bioactive Components of Magical Velvet Beans, Legume Crops - Prospects, Production, and Uses. Intech Open. 92124/chapters/72106 (2020).
18. Natarajan K, Narayanan N, Ravichandran N. Review on "Mucuna"-The wonder plant. International Journal of Pharmaceutical Science Rev Res. 17(1):86-93 (2012).
19. Suresh S, Elumalai Prithiviraj and Seppan Prakash. Effect of *Mucuna pruriens* on oxidative stress mediated damage in aged rat sperm. Volume 33 Issue 1: Pages 22 – 32 (2009).
20. Pathania R, Chawla P, Khan H, Kaushik R, Khan M A. An assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: a natural food legume. 3 Biotech 10 :261 (2020).

21. Rajeshwar Y, Gupta M, Mazumder UK. Antitumor and in vivo antioxidant status of *Mucuna pruriens* (Fabaceae) seeds against Ehrlich ascites carcinoma in Swiss albino mice. *Iranian J Pharm Ther*. 4: 46-53 (2005).
22. Chitra Kalyanaraman. Infertility Treatment in male rats by methanol extract of Mucuna pruriens. Journal of Cell and Tissue Research Vol. 22(2): 7255-7261 (2022).
23. World Health Organization. Laboratory manual for the examination of human semen and sperm cervical mucus interaction 4th. New York: Cambridge University Press (1999).
24. Latchoumycandane C, Mathur PP. Effects of vitamin E on reactive oxygen species-mediated 2,3,7,8-tetrachlorodi-benzo-p-dioxin toxicity in rat testis. *J Appl Toxicol*.22(5):345-51 (2002).
25. Yokoi K, Imai T, Shibata A, Hibi Y, Kikumori T, Funahashi H, Nakao A. Mild persistent hypercalcitoninemia after total thyroidectomy in patients with papillary thyroid carcinoma. *J. Ethnopharmacol*. 62: 183-193 (2003).
26. Sonmez S, Kirilmaz L, Yucesoy M, Yücel B, Yilmaz B. The effect of bee propolis on oral pathogens and human gingival fibroblasts. *J Ethnopharmacol*. Dec 1;102(3):371-6 (2005).
27. Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp RW Jr, Letz G, Malling HV, Topham JC, Whorton MD. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat Res* 115(1):73-148 (1983).
28. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld L J. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil*.70(1):219-28 (1984).
29. Yamamoto M, Takeuchi N, Kotani S, Kumagai A. Effects of glycyrrhizin and cortisone on cholesterol metabolism in the rat. *Endocrinol Jpn*.;17(5):339-48 (1970).
30. Filho DW, Torres MA, Bordin AL B. Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. *Mol Aspects Med*. 25(1-2):199-210 (2004).
31. Amin KMY, Khan MN, Rahman SZ, Khan NA. Sexual function improving effect of *Mucuna pruriens* in sexually normal male rats. *Fitoterapia*. 67: 53-8 (1996).
32. Rajesh Kumar T and Muralidhara. Oxidative stress response of rat testis to model prooxidants in vitro and its modulation. Toxicology in Vitro. Volume 16, Issue 6: 675-682 (2002).
33. Schrade, S M. Man and the workplace; Assessing his reproductive health. Chemical Health and Safety 2013, 11-16 (2003).
34. Takahashi and Oishi. Testicular toxicity of dietary 2,2-bis(4-hydroxyphenyl) propane (bisphenol A) in F344 rats. *Archives of Toxicology* . volume 75: pages42–51 (2001).
35. Meistrich ML, Wilson G, Huhtaniemi I. Hormonal treatment after cytotoxic therapy stimulates recovery of spermatogenesis. *J. Am. Coll. Toxicol*. 8: 551–567 (1989).
36. Perreault SD. The mature spermatozoa as a target for reproductive toxicants. In: Boekelheide K, Chapin RE, Hoyer PB, Harris C, eds. Comprehensive toxicology, vol. 10. *Reproductive and Endocrine Toxicology*:165–79 (1997).
37. Aitkin R J, Clarkson J S, Fishel S. Generation of reactive oxygen species, lipid peroxidation and human sperm function. *Biol. Reprod* .41 :183-187 (1989).
38. Shami PJ, Maciag AE, Eddington JK, Udupi V, Kosak KM, Saavedra JE, Keefer LK. JS-K, an arylating nitric oxide (NO) donor, has synergistic anti-leukemic activity with cytarabine (ARA-C). *Leuk Res* 33(11):1525-9 (2009).
39. Yousef MI, El-Morsy AM, Hassan MS. Aluminium -induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: protective role of ascorbic acid. *Toxicology*. 5;215(1-2):97-107 (2005).
40. Lafuente A, Marquez N, Pousada Y, Pazo Dm Esquifino AL. Possible estrogenic and or antiandrognic effects of methoxychlor on prolactin release in male rats. Arch Toxicol .74 (4-5):270-275 (2000).

41. Zhai J, Lanclos K D, Abney TO. Estrogen receptor messenger ribonucleic acid changes during Leydig cell development. *Biol Reprod*. 55(4):782-8 (1996).
42. Warren DW, Wisner Jr JR, Ahmad N. Effects of 1,2-dibromo-3- chloropropane on male reproductive function in the rat. *Biol Reprod*; 31:454–63 (1984).
43. Ahmad N, Wisner JR, Warren DW. Morphological and biochemical changes in the adult male rat reproductive system following long-term treatment with 1,2-dibromo-3-chloropropane. *Anat Rec*, 222: 340–9 (1988).
44. Yoshida S, Yamada H, Sugawara I, Takeda K. Effect of dibromochloropropane(DBCP) on the hormone receptors of the male rat reproductive system. *Biosci Biotechnol Biochem*; 62:479–83 (1998).
45. Mantovani A. Hazard identification and risk assessment of endocrine disrupting chemicals with regard to developmental effects. Toxicology. 181-182:367-370 (2002).
46. Walton S, Cunliffe W J, Keczkes K, Early A S, Mcgarrigle H H G, Katz M, Reese R. A Clinical, ultrasound and hormonal markers of androgenicity in acne vulgaris. BJD Volume133, Issue2 August Pages 249-253 (1995).
47. Pease W, Vandenberg J, Hooper K. Comparing alternative approaches to establishing regulatory levels for reproductive toxicants: DBCP as a case study. *Environ Health Perspect*. Feb; 91:141-55 (1991).
48. Fiorini C, Tilloy-Ellul A, Chevalier S, Charuel C, Pointis G. Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants. *Reprod Toxicol*. 18:413–21 (2004).
49. Pectasides D, Pectasides M, Farmakis D, Nikolaou M, Koumpou M, Kostopoulou V, Mylonakis N. Testicular Function in Patients with Testicular Cancer Treated with Blieomycin- Etoposide – Carboplatin (BEC 90) combination chemotherapy. European Urology. Volume 45, Issue 2, Pages 187-193 (2004).
50. Doshi PK, Chhaya NA, Bhatt MA. Bilateral subthalamic nucleus stimulation for Parkinson's disease. *Neurol* India. 51(1):43-8 (2003).

This page is intentionally left blank