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

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Abstract

Index terms—

1 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.

London Journal of Research in Science: Natural and Formal 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)(12)(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.

2 II. MATERIALS AND METHODS

3 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±2ºC) with 12 h light and 12 h dark exposure. The rats received a standard rat pellet diet and water ad libitum.

4 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).

5 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

6 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 ? C, 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.

7 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.

8 III. SPERM ANALYSIS

9 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.

10 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.

11 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).

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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 Trishydroxymethyl aminomethane, 0.50 g of glucose, 1.99 g of citric acid and 100 ml of distilled water) on a prewarmed (37ºC) 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).

12 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).

13 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.

14 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.

15 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.

16 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.

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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 ? 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.

18 Sperm Count

The results demonstrated the decrease in sperm count of rats under stress.

19 IV. RESULTS

20 Bodyweight and Organ weight

21 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 Testosterone, luteinizing hormone and follicle stimulating hormone levels in the male experimental rats were observed (Fig. **??**a,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. 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. (42)(43)(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, **??**iorini 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.

22 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.

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Figure 1:

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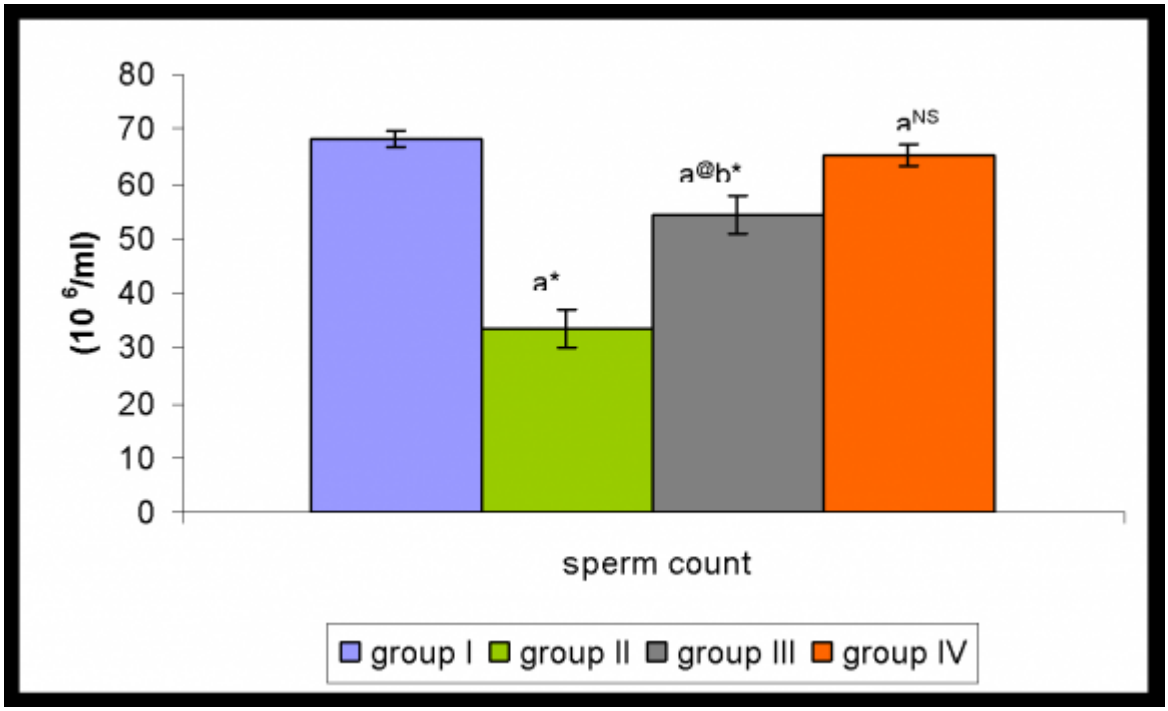
Figure 2: Fig 1 :

Figure 3: Fig



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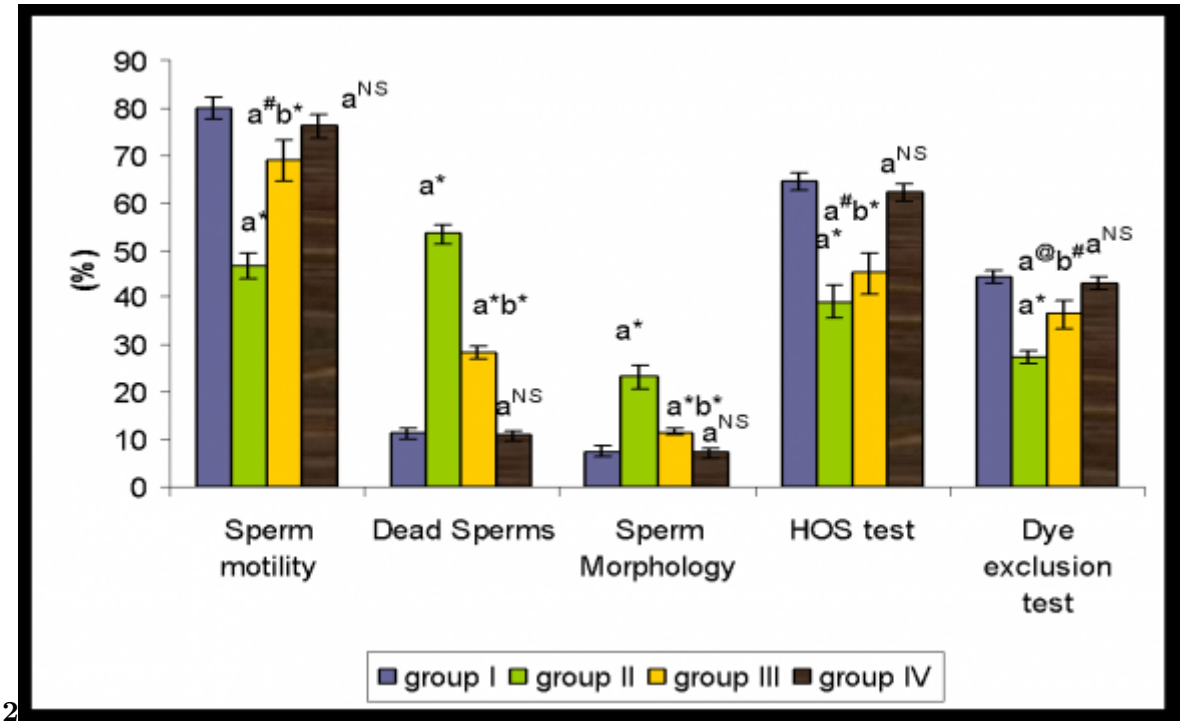

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Figure 5: Fig 2 :

1

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

NSValues 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 $p<0.05$.

Figure 6: Table 1 :

Figure 7:

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[Virant-Klun et al. ()] , Irma Virant-Klun , Senka Imamovic-Kumalic , Bojana Pinter . *Endocrine-Disrupting Chemicals (Bisphenols, Phthalates, and Parabens) with Human Semen Quality Antioxidants* 2022. 11 (8) p. 1617.

[Walton et al. (1995)] 'A Clinical, ultrasound and hormonal markers of androgenicity in acne vulgaris'. S Walton , W J Cunliffe , K Keczkes , A S Early , H Mcgarrigle , G Katz , M Reese , R . *BJD* August. 1995. 133 (2) p. .

[Yousef et al. ()] 'Aluminium -induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: protective role of ascorbic acid'. M I Yousef , A M El-Morsy , M S Hassan . *Toxicology* 2005. 5 (1-2) p. .

[Kavitha ()] 'Amazing bean Mucuna pruriens: A comprehensive review'. Thangamani C Kavitha . *Journal of Medicinal Plants Research* 2014. 8 p. .

[Pathania et al. ()] 'An assessment of potential nutritive and medicinal properties of Mucuna pruriens: a natural food legume'. R Pathania , P Chawla , H Khan , R Kaushik , M A Khan . *London Journal of Research in Science: Natural and Formal* 2020. 10 (3) p. 261. (Biotech)

[Wyrobek et al. ()] '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'. A J Wyrobek , L A Gordon , J G Burkhart , M W Francis , R W Kapp , Jr , G Letz , H V Malling , J C Topham , M D Whorton . *Mutat Res* 1983. 115 (1) p. .

[Rajeshwar et al. ()] 'Antitumor and in vivo antioxidant status of Mucuna pruriens (Fabaceae) seeds against Ehrlich ascites carcinoma in Swiss albino mice'. Y Rajeshwar , M Gupta , U K Mazumder . *Iranian J Pharm Ther* 2005. 4 p. .

[Doshi et al. ()] 'Bilateral subthalamic nucleus stimulation for Parkinson's disease'. P K Doshi , N A Chhaya , M A Bhatt . *London Journal of Research in Science: Natural and Formal* 2003. 51 (1) p. . (Neurol India.)

[Suryawanshi et al. ()] *Bioactive Components of Magical Velvet Beans, Legume Crops -Prospects, Production, and Uses*, Prajakta P Suryawanshi , Kamble , A Vishwas , Jyoti P Bapat , Jadhav . 2020. (Intech Open. 92124/chapters/72106)

[Reed et al. ()] 'Circular dichroic investigations of secondary structure in synthetic peptide inhibitors of cAMP-dependent protein kinase: a model for inhibitory potential'. J Reed , V Kinzel , H C Cheng , D A Walsh . *Biochemistry* 1987. 1 (24) p. .

[Pease et al. (1991)] 'Comparing alternative approaches to establishing regulatory levels for reproductive toxicants: as a case study'. W Pease , J Vandenberg , K Hooper . *Environ Health Perspect* Feb. 1991. 91 p. .

[Jeyendran et al. ()] 'Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics'. R S Jeyendran , H H Van Der Ven , M Perez-Pelaez , B G Crabo , L Zaneveld . *J Reprod Fertil* 1984. 70 (1) p. .

[Bernard et al. ()] 'Dichlorodiphenyltrichloroethane impairs follicle stimulating hormone receptor-mediated signaling in rat Sertoli cells'. L Bernard , N Martinat , C Lécureuil . *Reprod Toxicol* 2007. 23 p. .

[Yoshida et al. ()] 'Effect of dibromochloropropane(DBCP) on the hormone receptors of the male rat reproductive system'. S Yoshida , H Yamada , I Sugawara , K Takeda . *Biosci Biotechnol Biochem* 1998. 62 p. .

[Suresh et al. ()] *Effect of Mucuna pruriens on oxidative stress mediated damage in aged rat sperm*, S Suresh , Elumalai Prithiviraj , Seppan Prakash . 2009. 33 p. .

[Warren and Jr ()] 'Effects of 1,2-dibromo-3-chloropropane on male reproductive function in the rat'. D W Warren , Wisner Jr , JR , AhmadN . *Biol Reprod* 1984. 31 p. .

[Perry ()] 'Effects of environmental and occupational pesticide exposure on human sperm: a systematic review'. M J Perry . *Hum Reprod* 2008. 14 p. .

[Yamamoto et al. ()] 'Effects of glycyrrhizin and cortisone on cholesterol metabolism in the rat'. M Yamamoto , N Takeuchi , S Kotani , A Kumagai . *Endocrinol Jpn* 1970. 17 (5) p. .

[Latchoumycandane and Mathur ()] 'Effects of vitamin E on reactive oxygen species-mediated 2,3,7,8-tetrachlorodi-benzo-p-dioxin toxicity in rat testis'. C Latchoumycandane , P P Mathur . *J Appl Toxicol* 2002. 22 (5) p. .

[Zhai et al. ()] 'Estrogen receptor messenger ribonucleic acid changes during Leydig cell development'. J Zhai , K D Lanclos , T O Abney . *Biol Reprod* 1996. 55 (4) p. .

[Whorton et al. ()] 'Function in DBCP exposed pesticide workers'. D Whorton , R M Krauss , S Marshall , T H Milby , Stubbs . *Lancet, ii* 1977. p. .

[Aitkin et al. ()] 'Generation of reactive oxygen species, lipid peroxidation and human sperm function'. R J Aitkin , J S Clarkson , S Fishel . *Biol. Reprod* 1989. 41 p. .

[Mantovani ()] 'Hazard identification and risk assessment of endocrine disrupting chemicals with regard to developmental effects'. A Mantovani . *Toxicology* 2002. p. .

[Meistrich et al. ()] 'Hormonal treatment after cytotoxic therapy stimulates recovery of spermatogenesis'. M L Meistrich , G Wilson , I Huhtaniemi . *J. Am. Coll. Toxicol* 1989. 8 p. .

[Nykolaichuk et al. ()] *Impact of Environmental Factors on Male Reproductive Health Wiad Lek*, P Nykolaichuk , Roksolana , S Oleksandr , Fedoruk , V Volodymyr , Vizniuk . 2020. 73 p. .

[Sarah et al. ()] 'Impact of environmental toxin exposure on male fertility potential'. Krzastek Sarah , C , Jack Farhi , Marisa Gray , Ryan P Smith . *Transl Androl Urol* 2020. 9 (6) p. .

[Kalyanaraman ()] 'Infertility Treatment in male rats by methanol extract of Mucuna pruriens'. Chitra Kalyanaraman . *Journal of Cell and Tissue Research* 2022. 22 (2) p. .

[Shami et al. ()] 'JS-K, an arylating nitric oxide (NO) donor, has synergistic anti-leukemic activity with cytarabine (ARA-C)'. P J Shami , A E Maciag , J K Eddington , V Udupi , K M Kosak , J E Saavedra , L K Keefer . *Leuk Res* 2009. 33 (11) p. .

[Laboratory manual for the examination of human semen and sperm cervical mucus interaction 4th ()] *Laboratory manual for the examination of human semen and sperm cervical mucus interaction 4th*, 1999. New York: Cambridge University Press.

[Agarwal et al. ()] 'Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility'. A Agarwal , N Parekh , Panner Selvam , MK . *World J Mens Health* 2019. 37 (3) p. .

[Schrade ()] *Man and the workplace; Assessing his reproductive health. Chemical Health and Safety*, S Schrade . 2013. 2003. p. .

[Yokoi et al. ()] 'Mild persistent hypercalcitoninemia after total thyroidectomy in patients with papillary thyroid carcinoma'. K Yokoi , T Imai , A Shibata , Y Hibi , T Kikumori , H Funahashi , A Nakao . *J. Ethnopharmacol* 2003. 62 p. .

[Ahmad et al. ()] 'Morphological and biochemical changes in the adult male rat reproductive system following long-term treatment with 1,2-dibromo-3-chloropropane'. N Ahmad , J R Wisner , D W Warren . *Anat Rec* 1988. 222 p. .

[Fung et al. ()] 'Mucuna pruriens Linn. seed extract pretreatment protects against cardiorespiratory and neuromuscular depressant effects of Naja sputatrix (Javan spitting cobra) venom in rats'. S Y Fung , N H Tan , S M Sim , E Marinello , R Guerranti , J C Aguiyi . *Indian Journal of Experimental Biology* 2011. 49 (4) p. .

[Herman ()] 'Neural control of chronic stress adaptation'. J P Herman . *Front Behav Neurosci* 2013. 7 p. 61.

[Kumar ()] 'Oxidative stress response of rat testis to model prooxidants in vitro and its modulation'. Rajesh Kumar , T , Muralidhara . *Toxicology in Vitro* 2002. 16 (6) p. .

[Kelce et al. ()] 'Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist'. W R Kelce , C R Stone , S C Laws . *Nature* 1995. 375 p. .

[Lafuente et al. ()] 'Possible estrogenic and or antiandrognic effects of methoxychlor on prolactin release in male rats'. A Lafuente , N Marquez , Y Pousada , Pazo Dm Esquifino , AL . *Arch Toxicol* 2000. 74 (4-5) p. .

[Mohamadpour et al. ()] 'Protective effects of curcumin co-treatment in rats with establishing chronic variable stress on testis and reproductive hormones'. M Mohamadpour , A Noorafshan , S Karbalay , T Talaei , E Aliabadi . *Int J Reprod Biomed* 2017. 15 (7) p. .

[Natarajan et al. ()] 'Review on "Mucuna"-The wonder plant'. K Natarajan , N Narayanan , N Ravichandran . *International Journal of Pharmaceutical Science Rev Res* 2012. 17 (1) p. .

[Fiorini et al. ()] 'Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants'. C Fiorini , A Tilloy-Ellul , S Chevalier , C Charuel , G Pointis . *Reprod Toxicol* 2004. 18 p. .

[Amin et al. ()] 'Sexual function improving effect of Mucuna pruriens in sexually normal male rats'. Kmy Amin , M N Khan , S Z Rahman , N A Khan . *Fitoterapia* 1996. 67 p. .

[Filho et al. ()] 'Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury'. D W Filho , M A Torres , Al B Bordin . *Mol Aspects Med* 2004. 25 (1-2) p. .

[Pectasides et al. ()] 'Testicular Function in Patients with Testicular Cancer Treated with Blieomycin-Etoposide -Carboplatin (BEC 90) combination chemotherapy'. D Pectasides , M Pectasides , D Farmakis , M Nikolaou , M Koumpou , V Kostopoulou , N Mylonakis . *European Urology* 2004. 45 (2) p. .

[Takahashi ()] 'Testicular toxicity of dietary 2,2-bis(4-hydroxyphenyl) propane (bisphenol A) in F344 rats'. Oishi Takahashi . *Archives of Toxicology* 2001. 75 p. .

[Sonmez et al. ()] 'The effect of bee propolis on oral pathogens and human gingival fibroblasts'. S Sonmez , L Kirilmaz , M Yucesoy , B Yücel , B Yilmaz . *J Ethnopharmacol. Dec* 2005. 1 (3) p. .

[Lampariello et al. ()] 'The magic velvet bean of Mucuna pruriens'. L R Lampariello , A Cortelazzo , R Guerranti , C Sticozzi , G Valacchi . *Journal of traditional and complementary medicine* 2012. 2 (4) p. .

[Perreault ()] 'The mature spermatozoa as a target for reproductive toxicants'. S D Perreault . *Reproductive and Endocrine Toxicology*, K Boekelheide , R E Chapin , P B Hoyer , C Harris (eds.) 1997. 10 p. .

[Teitelbaum ()] 'The toxicology of 1,2-dibromo-3-chloropropane (DBCP): a brief review'. D T Teitelbaum . *Int J Occup Environ Health* 1999. 5 p. .

[Linh et al. ()] 'Time-course changes of steroidogenic gene expression and steroidogenesis of rat Leydig cells after acute immobilization stress'. Yuank Linh , Zhouh-Y , But , Suh , Lius , Zhuq , Wangy , Huy , Shany . *Int J Mol Sci* 2014. 15 (11) p. .