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Orignal Article

INFERTILITY TREATMENT IN MALE RATS BY METHANOL-EXTRACT OF *MUCUNA PRURIENS*

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Abstract: ***Methanolic seed extract of Mucuna pruriens (M. pruriens) is identified as an herbal medicine for improving fertility-related problems. Hence the present study deals to identify the potential effect of M. pruriens on testicular enzymes and sperm parameters in male rats exposed to 1, 2-dibromo 3 chloropropane (dbcp). Male sprague-dawley rats were divided into four groups. Group 1: control received 0.9% saline, group 2: single dose of 50mg/kg 1, 2-dibromo 3 chloropropane (dbcp) for 30 days, Group 3: 50mg/kg of 1, 2-dibromo 3 chloropropane (dbcp) for 30 days after that 200mg/kg/day M. pruriens for 45 days. Group 4: 200mg/kg/day bwof M. pruriens for 45 days. Study shows sperrm concentration, motility, morphology, and vitality in groups 3 and 4 treated with M. pruriens significantly increased. In group 2 dbcp-treated rats, it was found that testicular marker enzymes as acid phosphatase (ACP), alkaline phosphatase (ALP), ß-D glucuronidase, and y-glutamyl transpeptidase (y-gt) are enhanced while lactate dehydrogenase (LDH) is decreased. It is concluded that M. pruriens is a potent natural plant material for treating male infertility. Based on the findings, the methanol extract of M. pruriens significantly improves sperm parameters and testicular marker enzymes in dbcp treated rats.***

Keywords: **1, 2-dibromo 3 chloropropane** , Sperm motility,

INTRODUCTION

An increase in synthetic products on male reproductive health is much concerned in recent years [1]. The scientific and medical literature described a variety of environmental factors that influence male infertility [2]. Organic chemical solvents, pesticides, heavy metals, and other persistent organic pollutants are among the environmental factors being studied by scientists. Among these agents, special concerns have been raised by endocrine disrupting chemicals, which are hormonally active compounds that can interfere with the normal reproductive system [3].

The public and scientific communities are becoming increasingly concerned about the possibility that non-degradable environmental contaminants may

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endanger fertility in humans [4]. After one year of unprotected intercourse, a male is said to be infertile if he is unable to impregnate his partner.Human Infertility is becoming a social issue, encompassing feelings of rage, depression, anguish, guilt, and embarrassment [5]). Male sensitivity to spermatogenesis has been extensively studied all over the world. Rather than sperm morphology, sperm count, and motility are the first and most important predictors of fertility potential. Therefore, it is crucial to identify these exogenous factors to decrease exposure and subsequently enhance sperm quality.

Pesticides are ever-present contaminants of our environment and have been found in air, soil, water, and human and animal tissue samples all over the world. Certain substances are considered environmental contaminants that disturb the endocrine system in the human body resulting in male and female infertility [6,7]. 1, 2 Dibromo 3 chloropropane (DBCP), is one such organochlorine pesticide used widely to control Agricultural and Domestic pests. Potashnik *et al.*, [8] observed that the workers exposed to DBCP suffer from Azoospermia, Oligospermia as well as inhibiting effect on spermatogenesis.According to Daoud *et al.* [9] pesticide exposure is associated with a significantly increased risk of Asthenozoospermia and Necrozoospermia. Therefore, in the present investigation DBCP was selected to induce reproductive toxicity in male rats.

Mucuna pruriens (M. pruriens) is an annual herbaceous twinning plant native to India that is well known for its itching properties [10]). A review of the literature reveals that the medicinal properties of *M. pruriens* have a positive effect on sperm count and motility [11]. Misra and Wagner [12] reported that *M. pruriens* contains a variety of other bioactive substances, including tryptamine, alkylamines, steroids, flavonoids, coumarins, cardenolides, oleic acid, linoleic acid, and palmitic acid [13]. Antioxidant [14], Antivenomic [11], Antiepileptic, Antineoplastic, Antimicrobial [15), and Neuroprotective activities [16] are also found in *M. pruriens* seeds. The seed has been used internally to treat Parkinson's disease, edema, impotency,and Nervous Disorder [17].Though, *M. pruriens* has been extensively reported to possess various biological activities, there is a paucity of information against DBCP induced testicular toxicity in rats. Therefore, the present investigation was aimed to study the efficacy of *M. pruriens* against DBCP induced testicular toxicity in rats.

MATERIALS AND METHODS

***Mucuna Pruriens* seeds:** The seeds of *M. pruriens* were purchased frommarket during the month of January 2009. The seeds were authenticated by the Chief Botanist, Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC) Chennai, India. A voucher specimen has been deposited at the Department of Pharmacology and Environmental Toxicology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai, Tamilnadu, India.

Preparation of the methanolic extract 0f *M. Pruriens*: The seeds of *M. pruriens*were shade dried and then coarsely powdered. A known weight of the 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 residue by using a water bath. The yield of the total methanolic extract was 13%w/w and then the extract was stored in a refrigerator for further studies.

A**nimals:** Healthy adult male Sprague- Dawley rats (8 weeks old), weighing between 160-220g were used in the present study. They 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). The rats received a standard rat pellet diet and water *ad libitium*. The rats were housed under conditions of controlled temperature (26±2şC) with 12 h light and 12 h dark exposure.

Mounting behavior: The mounting behavior test was carried out to check the fertility of the male rats before starting the experimental Design.Male and Female rats cohabitated to check their sexual capability. The copulatory plug in the female rats confirms copulation.

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 intragastrically for 30 days. Group III - After 1,2 Dibromo 3 chloropropane (dbcp) treatment, methanolic extract of *M. pruriens* (200mg/kg/day body weight) was administered intragastrically 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. [55] with ± 200 mg to confirm effective concentration.

Collection of samples: The animals were sacrificed by cervical dislocation under mild ether anesthesia after the experimental period. The testes and epididymis were dissected out and washed with saline before being blotted and stored at -20 C until further analysis. In Tris - HCl buffer (0.1M; pH 7.4), the tissues were homogenized. The supernatants were tested for biochemical parameters.

Epididymal sperm concentration and motility: Sperm morphology was evaluated by calculating the percentage of normal and abnormal forms using the Diff - Quick staining method [18]. For morphologic studies, sperm smears were prepared and stained using the papanicolaou stain according to the method [19]. Using a hemocytometer and a modification of the method [20] epididymal sperms were counted. The sperm vitality or hypo-osmotic swelling test was carried out exactly as described [21]. Sperm vitality was determined using Eosin-nigrosin staining.

Testicular marker enzyme assay: The supernatant was used for the testicular marker enzyme assay escribed below. The King method [22]) was used to measure lactate dehydrogenase. The activity of ß-glutamyl transpeptidase (y- GT) was determined using the method [23]. The method of Delvin and Gianetoo [24] was used to calculate ß-D Glucuronidase activity. Bergmeyer's method [25] was used to estimate acid and alkaline phosphatases.

Statistical analysis: The mean and standard deviation were used to present the data (SD). To compare the means of different Groups of using SPSS. 7.5 student versions, a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used.

RESULTS

Sperm morphology: The results of sperm characteristics of the present study are presented in figures 1 and 2, respectively. Figure 1 shows the sperm count in control and 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. After *M. pruriens* treatment, the sperm count increased significantly in Group III animals and was comparable to that of Group II toxicity-bearing animals. However, no variances were observed in Group IV *M. pruriens* alone treated male rats.

Figure 2 shows sperm motility, morphology, and vitality of experimental rats. Same as sperm count the sperm motility, vitality by HOS and dye exclusion test decreases in Group II dbcp treated male rats, which was significantly reverted to near normal in *M. pruriens* extract-treated Group III animals. Whereas the dead and morphology of sperm in Group 2 toxicity bearing animal were significantly increased when compared to Group I control male rats. These abnormalities were re-stored in Group III *M. pruriens* extract-treated animals.Whereas there were no noticeable changes in Group IV *M. pruriens* alone treated animals when compared to Group I control animals.

Testicular marker enzymes: Table 1 shows the Marker enzymes in testis of control and experimental rats. In Group II toxicity bearing animals ACP, ALP, ß-Glucuronidase, and y-GT was increased and LDH was decreased when compared to Group 1 control animals. On the other hand, a significant increase in LDH activity and in contrast to this a remarkable decrease in the ACP, ALP, ß-D glucuronidase and y-GT were observed in group III *M. pruriens* extract treated animals and was comparable to that of group 2 toxicity bearing animals. No changes were observed in group IV methanol seed extract alone treated animals when compared to group I control animals.

DISCUSSION

Reproductive toxicity can be defined as a dysfunction of the reproductive system induced by chemical agents and numerous toxic agents. Male infertility has been linked to sperm count and percentage of abnormal sperm in animals [27]. DBCP is one such

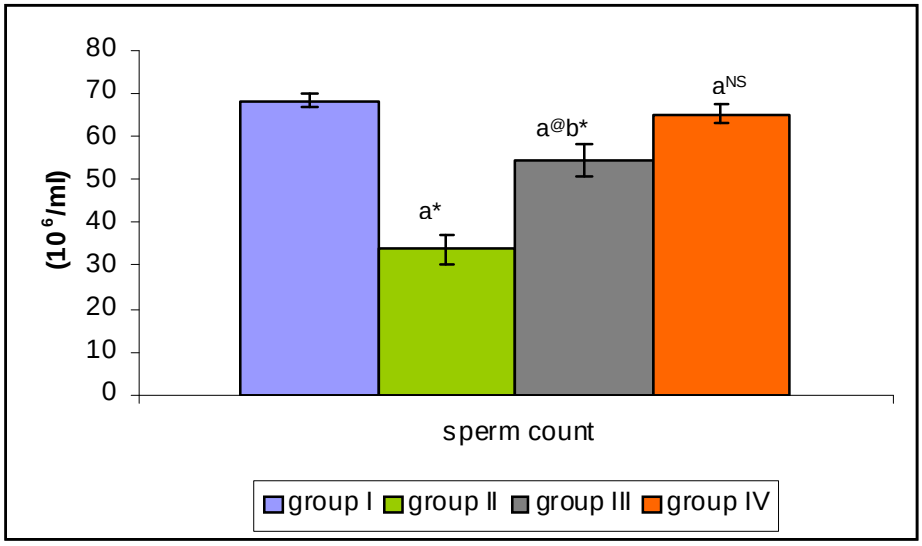

Fig 1: Efficacy of *M. pruriens* on sperm count in control and experimental animals. Each value represents mean ± SD are average of data for 6 animals. a – Group II, III, IV compared with Group I, b – Group III compared with Group II. Statistical significance is indicated as $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

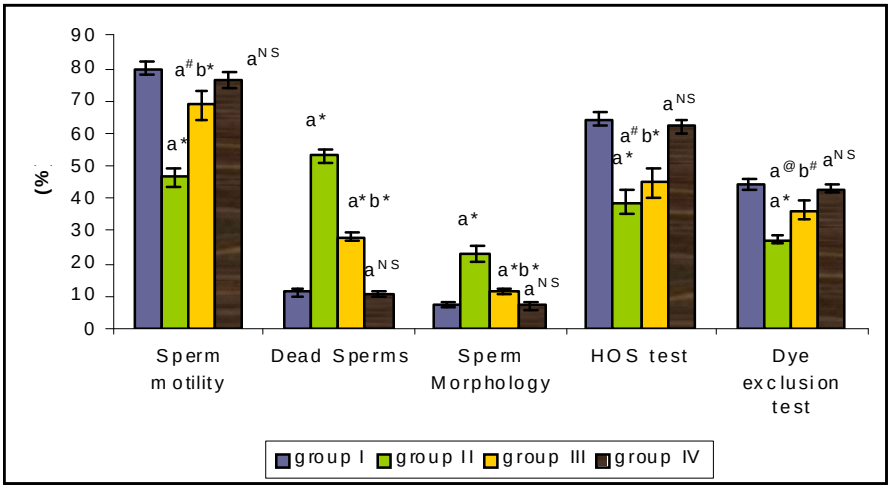

Fig : 2 Efficacy of *M. pruriens* on sperm motility, morphology, and vitality of control and experimental animals. Each value represents mean ± SD are average of data for 6 animals. a – Group II, III, IV compared with Group I, b – Group III compared with Group II. Statistical significance is indicated as $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

Table 1: Activities of marker enzymes in the testis of control and experimental animals. 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 , $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

Parameters	Group I (Control)	Group II (DBCP)	Group III (DBCP + *M. pruriens*)	Group IV (*M. pruriens*)
LDH(µ mole of pyruvate liberated/mg protein/min)	0.98±0.03	0.65±0.05a*	0.91±0.06a# b*	0.96±0.03a NS
γ-GT (µ mole of p-nitro aniline formed /mg protein/min)	14.86±0.49	27.22±1.97a*	15.64±0.95a#b*	14.81±0.41a NS
β-D Glucouronidase (µ mole of p-nitro phenol liberated /mg protein/h)	18.29±0.53	28.48±2.63a*	18.88±1.10a*b*	17.24±0.57a NS
ACP(µ mole of ρ-nitrophenol liberated /mg protein/min)	22.37±0.35	52.60±0.51a*	42.04±0.84a#b*	26.45±0.39a NS
ALP(µ mole of ρ-nitrophenol liberated /mg protein/min)	44.43±0.38	84.31±5.81a*	52.92±0.84a#b*	43.36±0.39a NS

pesticide, best known for its male reproductive toxicity with a poor response to currently available chemotherapeutic agents in humans [32].The sperm count in the epididymis is one of the most sensitive tests for evaluating spermatogenesis because it provides information on all stages of meiosis, spermiogenesis, and transition in the epididymis [33].

In the present study the administration of DBCP in Group II rats significantly reduced sperm count when compared to Group I control animals. High-dose toxicants may cross the blood-testis barrier, affecting spermatogenesis and harming specific components of the seminiferous epithelium. According to Ban et al. [34], evaluating the number and percentage of abnormal spermatozoa is a useful method for quantitatively identifying the negative effects on spermatogenesis in rats. In the current study increased sperm count was observed in Group III *M. pruriens* treated rats because the phytochemical constituents present in the *M. pruriens* extract suppress free radical-mediated sperm disturbances. The current study findings are consistent with previous reports [35,36].

Sperm motility is frequently used to diagnose chemically induced testicular toxicity. Sperm movement is important for sperm functional capacity and assessing sperm movement is thought to be useful for detecting or assessing male reproductive toxicity [37].

The process of spermatogenesis from germ cells is said to be extremely vulnerable to any form of oxidative stress [38]. The epididymis is known to play an important role in the development of motility, fertilizing ability, and storage of sperm in mammals. Previous research has linked high levels of reactive oxygen species to sperm motility [39-42]. When DBCP-treated rats (Group II) were compared to Group I control rats, there was a decrease in sperm motility and abnormal sperm morphology.

According to reports, the HOS test was used to determine the physiologic integrity of the sperm membrane [21]. This assay evaluates the functional integrity of the sperm membrane under hypo-osmotic conditions. According to the present study, DBCP exposure caused severe plasma membrane damage in the sperm due to free radical generation. *M. pruriens* administration stabilized and restored normal membrane potential due to the presence of glycosides, saponins, and sterols in the seed extract. According to Yousef et al. [43], active compounds such as glycosides and phenols provide significant protection against free radical-mediated plasma membrane damage.

Acid and alkaline phosphatases, lactate dehydrogenase and ß-D glucuronidase are the most specific and sensitive marker enzymes [29-31]. As a result, analyzing the marker enzymes may be useful in disease management. It is well known that marker enzymes are extremely sensitive and are released into the circulation when tissues are damaged [28]. LDH is widely distributed in the seminiferous tubules and germ cells, and is associated with spermatogenic cell and spermatozoa maturation, as well as spermatozoa energy metabolism [44]. In the current study, inhibiting LDH in DBCP-bearing Group II animals may cause spermatogenic cells to denaturalize. Because LDH appearance and activity are linked to spermatogenesis and male testicular development, decreased activity of this enzyme in DBCP-treated group II animals indicates a defect in spermatogenesis and testicular maturation, whereas *M. pruriens* on the other hand, increased the activity of this enzyme in Group III rats.This may be due to the various phytochemical constituents present in the seed extract. In this regard, Mucuna species are extremely effective at reducing free radicals, including lipid peroxides, and improving mitochondrial metabolic activity [45,46].

The enzymes y-GT and ß-D glucuronidase are regarded as indicators of Sertoli cell activity. These enzymes' activities vary inversely with the number of spermatozoa and their maturation [47]. An increase in y-GT activity is a symptom of testicular atrophy caused by xenobiotic damage to germ cells and Sertoli cells [48,49]. Fluid secretion into the seminiferous tubules, which transport spermatozoa into the testis, is also mediated by y-GT [47]. In the current study, DBCP-treated group II rats had higher levels of testicular y-GT, ß-D glucuronidase, ACP, and ALP when compared to control animals. The DBCP exposure stimulates the function of Sertoli cells, increasing their supportive role in the transport of spermatozoa into the testis and due to tubular cell dysfunction caused by increased protein levels.

In the current study, DBCP was found to have effects on testis enzyme activities. Phosphatases are non-specific phosphomonoesterase enzymes that hydrolase orthophosphate esters in alkaline or acidic conditions. The increased activities of ACP and ALP in tissues reflect the catabolism of substances absorbed from the lumen as well as the release of non-specific phosphatases from degenerating cells' lysosomes [50]. The activity of ALP is related to the mitosis of spermatogenic cells and glucose transport. ACP located in the lysosome of Leydig cells is involved in protein synthesis by the abduction of sex hormones. Changes in the activity of ALP and ACP may be used as an indicator of spermatogenesis function [51-53].

The elevated activities of ACP and ALP in DBCP-administered Group II animals suggest the rapid catabolism of the injured germ cells in the animals. Changes in the activity of testicular ALP and ACP of DBCP-treated rats also reflect testicular degeneration, which may be a consequence of suppressed testosterone. There is evidence that chemical toxicants alter testicular morphology, causing spermatogenesis to be disrupted [54]. On the other hand, treatment with *M. pruriens*in Group III rats suppressed the increased levels of these enzymes by possibly stabilizing the plasma membrane and it suggests the cytoprotective action of *M. pruriens* in preventing DBCP-induced testicular damage.

CONCLUSION

M. pruriens is not only a reproductive enhancer, but also an important natural material for the treatment of male infertility. This study shows the ability of methanolic extract of *M. pruriens* treat male infertility was confirmed, which yielded encouraging results and observations. Treatment with methanolic extract of *M. pruriens* seed results in increased sperm concentration, motility, and improved sperm quality.

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