https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(December 2024); 1752-1760

Research Article

Protective Effect of *Mucuna Pruriens* on Fluoxetine-Induced Testicular Abnormalities in Chronic Unpredictable Stress Model in Male Rats

Apeksha[1], Vasanthalaxmi K Rao[1*], Manjula S. D[1*], Mohan das [2], Rucha Dafale [1], Shobha Kamath[3], Pallavi L. C[1], Nanda Acharya[1]

[1]*Department of Physiology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka-576104, India.*
[2] *Department of Anatomy, Department of Basic Sciences, Manipal Academy of Higher Education, Manipal, Karnataka-576104, India.*
[3]*Department of Biochemistry, Kasturba Medical College (KMC), Manipal, Manipal Academy of Higher Education (MAHE), Manipal, Karnataka-576104, India.*

ABSTRACT
Fluoxetine, a popularly prescribed antidepressant. Long-term FLX use resulted in male infertility *Mucuna pruriens* is a potent medicinal plant that has been used as an adjunctive therapy for male infertility. To study the protective effect of *Mucuna pruriens* on fluoxetine induced testicular dysfunction in chronic unpredictable mild stress (CUMS) induced male rats, 30 Albino Wistar male rats weighing 180-250gm, aged around 3 months old were used. CUMS will be induced for the first 4 weeks. After confirming the depression, Animals were allocated into 5 groups of 6 animals each, control, CUMS, CUMS + fluoxetine, CUMS+ aqueous extract of *Mucuna prureins* and CUMS+ fluoxetine + aqueous extract *Mucuna pruriens* respectively.
At the end of the treatment period, the animals were sacrificed, testes, and epididymis were removed and weighed. One of the testes was used for histology studies, while the other testis was used for testicular Malondialdehyde and Glutathione (GSH) estimation. The cauda epididymis of the rats was used to assess sperm parameters like sperm count, and sperm motility. The aqueous extract of *Mucuna pruriens* showed a significant improvement in testicular weight, sperm count and motility, histopathological changes, and testicular MDA and GSH level in FLX-treated and depression induced rats. *Mucuna pruriens* shows an improvement in sperm count, motility, lipid peroxidation which indicates its protective effect against the fluoxetine, could be due to increased endogenous level of antioxidants levels and decreasing free radical level as *Mucuna pruriens* is rich in flavonoids.

KEYWORDS: *Mucuna pruriens,* Fluoxetine, chronic unpredictable stress, sperm count and motility

***Author for correspondence: Email:** *manjula.sd@manipal.edu, vlupadhya@manipal.edu*

Received: 25/11/2024 Acceptance: 01/12/2024

DOI: https://doi.org/10.53555/AJBR.v27i3.4526

INTRODUCTION

Depression is major mental disorder affecting 5% of adults and it is fourth leading cause of death worldwide (WHO, 2023). Depression is a common chronic recurrent mood disorder, observed more frequently at reproductive ages. By statistics, male infertility represents about 35% of infertilities. Drugs have been reported to play a role in the etiology of male infertility. Chronic stress is known to be a psychological factor that can cause many neuropsychiatric disorders, metabolic syndromes, and infertility (Marwaha et al., 2023)

In animals, unpredictable stressors have been shown to induce changes in behavioral parameters, including changes in locomotor and explorative behavior, impairment of feeding, drinking and sexual behavior (Bataineh and Daradka, 2007). Such behavioral changes are often seen in human psychiatric disorders. A regime of uncontrollable stress has been used extensively to model the deficits in motivation and reward. In DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition -American Psychiatric Association 1994), anhedonia (loss of interest or pleasure in events that usually would be enjoyed) is defined as a core symptom of depression (Gocmez SS et al.,2010).

Fluoxetine (FLX) is a selective serotonin reuptake inhibitor (SSRI), commonly prescribed medication for treating neurological problems like anxiety and depression. It has been shown that long-term ingestion of fluoxetine for 60 days caused a great decrease in spermatogenesis in seminiferous tubules of the testes (Monteiro et al., 2014). Sperm motility and density were also significantly reduced in cauda epididymis and testes of the treated group. The weights of reproductive organs (testes, epididymis, ventral prostrate, and seminal vesicles) decreased considerably (Kerr JB et al.,1992). The hormonal assay also showed a significant decrease in testosterone levels and FSH levels. Testicular cell population dynamics also demonstrated a decrease in the number of both primary and secondary spermatocytes and spermatids in the treatment group (Bataineh and Daradka, 2007).

Mucuna pruriens (MP) is also called velvet bean is a potent medicinal plant that has been used for a long time in tropical areas, especially in China and India, as an adjunctive therapy for Parkinson's disease, type 2 diabetes, and male infertility (Khan et al., 2017). *Mucuna pruriens* seed extract has been found to have pharmacological actions that include neuroprotection, anti-oxidative, anti-inflammatory, anti-hypertensive, anti-diabetes, and anti-venom properties (Lampariello et al., 2012, Sinha et al.,2018). It has been demonstrated that Mucuna pruriens seed enhances the antioxidant enzymes and semen quality in the seminal plasma of infertile men (Shukla et., 2010). Mucuna pruriens improved the testicular mitochondria function and protect the sperm DNA in experimental animals (Abarikwu et al.,2020). With these medicinal properties of MP, the present study is designed to understand whether the administration MP can ameliorate FLX-induced testicular toxicity, and improve sperm count, motility, testicular MDA, and GSH levels in stress-induced rats.

MATERIALS AND METHODS

Animals

Before the experiment started, approval from the Institutional Animal Ethical Committee (IAEC/KMC/104/2022) was acquired. We obtained 8 weeks old male albino Wistar rats from the Central Animal Research Facility at KMC in Manipal. The animals were kept in a light and dark cycle (12:12) under conventional settings, including a temperature of 28°C, 50% humidity, and other standardized elements. The rats were kept in polypropylene cages in three-person groups with bedding made of sterilized rice husk that was locally sourced. In addition to unlimited access to water, they were fed granules of conventional laboratory food made by VRK Nutritional Solutions in Pune, India.

Plant extract (Aqueous) preparation: Seeds of Mucuna pruriens was purchased from Anamaya Herbals, Udupi, after grinding Mucuna pruriens seeds, powder was soaked with distilled water and subsequently, aqueous seed extract was filtered, and filtrate was dried. Later, it was kept in the desiccator until use. (According to KMC pharmacology lab protocol).

Chronic unpredictable Mild Stress (CUMS) model was used to induce depression in rats. 30 male Wistar rats weighing around 150-250g were fed and housed with standard pelleted diet, water ad libitum under standard environmental conditions for the purpose of acclimatization for two weeks. Rats were exposed to the forced swimming stress test daily for 28 days (4 weeks). The forced swim test has been widely employed for inducing physical stress in experimental animals (Porsolt, et al., 1977, Nayantara et al., 2005). It was further modified by Nayantara et al which was used for this experiment (He et al., 2020). Rats of all groups except control group were put to swim in plastic tanks (measuring length 40cm, width 40cm and depth 60cm) containing tap water at a temperature (36 ± 20 C) and the water depth was set at >35cm such that the rodent would not rest by using its tail for support at the bottom of the water tank. The rat was assessed and stated to be exhausted when it would not rise to the surface to breathe within a time period of 7 seconds. At this point, the animal was removed from the tank (Suresh et al.,2013). The same procedure was repeated for all the rats, one after another, every day for 28 days. Along with the force swimming test each animal was exposed to one of the other stressors every day as it is given in table 1.1.

Monday	Food deprivation + forcedswim test	Rats are subjected to 12hours of food deprivation from 8am.
Tuesday	Water deprivation + forced swimtest	Rats are subjected to 12hours of water deprivation from 8am.
Wednesday	Wet cage + forced swim test	Rats are immersed in 200ml of water for 24hours.
Thursday	Tilted cage + forced swim test	Rats are subjected to cage tilting at 45degree along the vertical axisfor 24hours.
Friday	Alternating light and dark + forced swim test	Applied throughout over 12hours dark phase and first 4hours of light phase

Table No. 1.1 - Exposure of rats to different types of stimuli to induce CUMS

Each experimental week is divided into 5 days of exposure to the stressor in and 2 days of non-exposure. Rat behavior was evaluated at the conclusion of the 4-week using the forced swim test & the sucrose preference test to confirm the depression is induced or not.

EXPERIMENT DESIGN

The animals were allocated into 5 groups, 6 animals in each group. Group-1 - Saline water, Group-2– CUMS, Group-3 - CUMS+ fluoxetine, Group 4- CUMS+ Mucuna pruriens, Group -5 CUMS+ Fluoxetine + Mucuna pruriens,

Drug dosage: oral administration by using gavage, fluoxetine-10mg/kg body weight. Mucuna pruriens: 300mg/kg body weight. Duration of Treatment: 30 days (Lapyuneyong et al., 2022)

Collection and Storage of Samples: Animals were weighed, testes, and epididymis were dissected and removed 24 hours after the previous treatment. Both testes were removed from the body and weighed. Part of the testis was preserved in the formalin and used for histological studies, while the other was conserved in PBS (phosphate-buffered saline) and kept at -80°C for biochemical estimation.

Semen sample: The cauda epididymis of the rats was dissected out to make a homogenized solution with a pH of 7.2 in 1 ml of PBS and the solution was used to assess sperm parameters.

PARAMETERS ASSESSED

1. Body weight: At the end of the treatment period, the animals were weighed, and the measured body weight was given in grams.
2. Testes weight: After the animals were sacrificed & dissected, the testes were collected and weighed.
3. Sperm count: Immediately, 1:40 dilution of the 1 ml sample of the solution was prepared by adding PBS. A sample from this solution was charged to Neubauer Chamber. Except for the center RBC counting region, the sperm counting was carried out in accordance with the procedure using the Neubauer chamber in eight squares. The quantity of sperm in total was calculated. In millions/ml, the total concentration was calculated by multiplying the amount by 5x104 (Rekha et al., 2021)
4. Sperm motility: 200 spermatozoa that were counted in 5 randomly selected fields were categorized into non-motile, non-increasingly motile, and gradually motile spermatozoa by observation under a light microscope (400 magnification) based on the pattern of motility. (Bezerra et al., 2019, Aruwa et al., 20214)
5. Histopathological analysis of testis: To make the paraffinized blocks, testis preserved in formalin were used with gradational series of alcohol attention, cleaned by xylene, and embedded with liquid paraffin (56-600 C). Additionally, a semi-automatic rotary microtome was used to segment the blocks. Following further deparaffinization with xylene, pieces were then rehydrated with intermittent alcohol and rinsed with tap water. After washing, the stained tissue sections were dehydrated and mounted with dibutyl phthalate polystyrene xylene, and all sectioned tissues were examined under a light microscope. Next, all sections were stained with hematoxylin and eosin stain.

6. Biochemical analysis:

6a. Testicular MDA levels:A testis-based lipid peroxidation marker is called MDA. Initial homogenization of the testis was followed by a mixture of 30% trichloroacetic acid (TCA), 5 N hydrochloric acid (HCl), 2% thiobarbituric acid (TBA), and 0.5 N sodium hydroxide (NaOH) being added to the homogenate. This mixture was centrifuged for an additional 10 minutes at 10,000 rpm after being incubated at 90 degrees Celsius for 15 minutes. Using a 96-well plate and an ELISA plate reader, the supernatant's pink colour was evaluated, and its absorbance at 532 nm was calculated (Lynda et al., 2024). The amount of MDA present in the tissue was given as nmol/g.

6b. Testicular GSH levels:The testis homogenate was added to the same amount of 10% TCA and centrifuged for a further ten minutes at 10,000 rpm at room temperature. After thoroughly shaking, 0.1 ml of the supernatant was added to 2 ml of PBS, 0.5 ml of DTNB (0.2%), and 0.4 ml of distilled water. GSH was determined using this mixture, where GSH reacts with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to produce a yellow chromophore. This was identified at 412 nm by an ELISA plate reader (Beeder et al., 2020)

Statistical analysis: Graph Pad Version 8 software was used to extensively analyze the data using one-way ANOVA (analysis of variance). A p-value of 0.05 with a 95% confidence interval was regarded as statistically significant for all measurements. Values are expressed as a metric called standard error of the mean (SEM).

RESULTS

Body weight: Body weight of depressed animals did not vary when compared to control group and Body weight of treatment group did not vary significantly when compared with rats treated with CUMS+ fluoxetine. (Fig No-2.1)

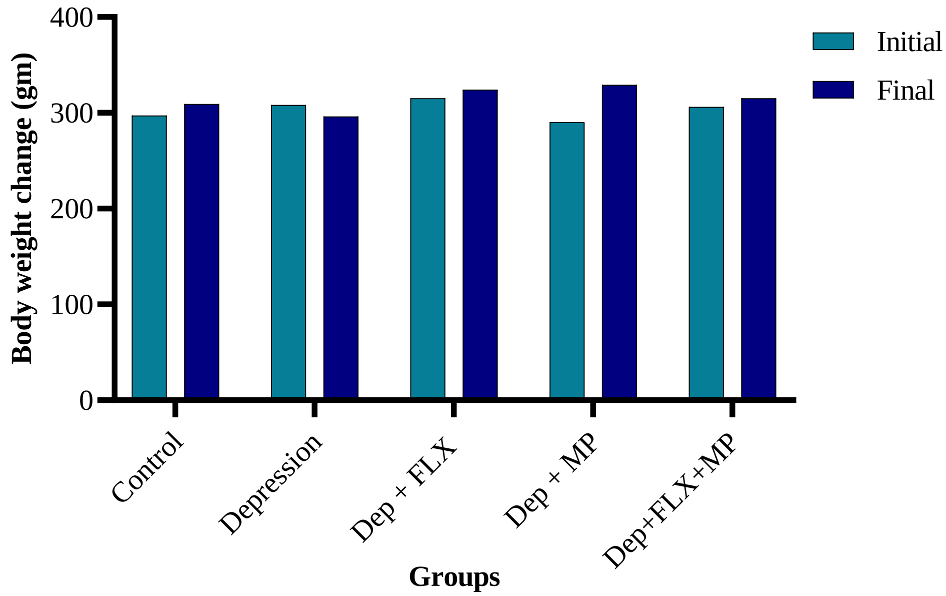


Fig no 2.1- change in body weight in control, depression, depression+FLX, depression +MP and Depression+MP+FLX groups of rats

Testes weight

CUMS group has shown significant decrease ($p < 0.05$) in the testes weight in comparison to the control group & rats treated with CUMS+FLX shown significant decrease($p<0.01$) in testes weight when compared with control group. Rats treated with CUMS+ MP & CUMS+FLX+MP have shown a significant increase ($p<0.01$) & ($p<0.05$) respectively inthe testes weight when compared to the rats treated with CUMS +FLX. (Fig no 2.2)

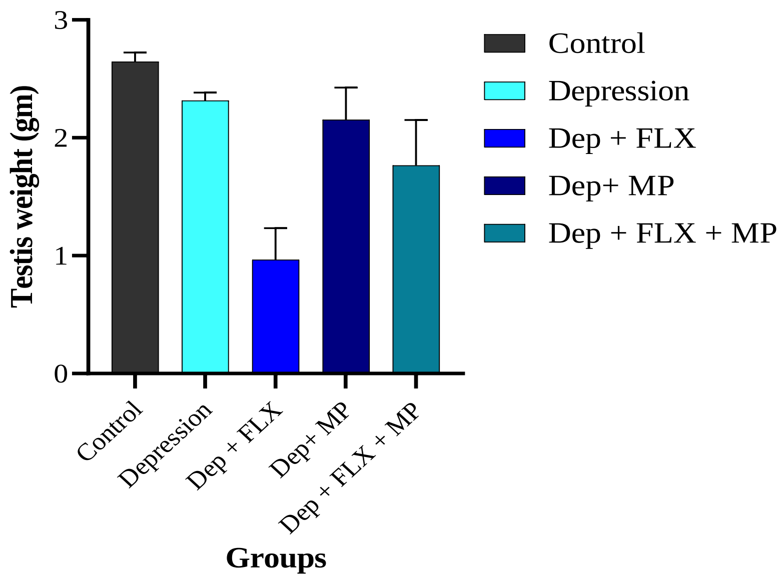


Fig no 2.2- change in testis weight in control, depression, depression+FLX, depression +MP and Depression+MP+FLX groups of rats

Sperm count

(Fig no 2.3) CUMS group has shown significant decrease ($p < 0.05$) in the sperm count in comparison to the control group & rats treated with CUMS+FLX shown significant decrease ($p<0.01$) in sperm count when compared with control group. Rats treated with CUMS+ MP &CUMS+FLX+MP have shown a significant increase ($p<0.05$) & ($p< 0.01$) respectively in the sperm count when compared to the rats treated with CUMS+ FLX.

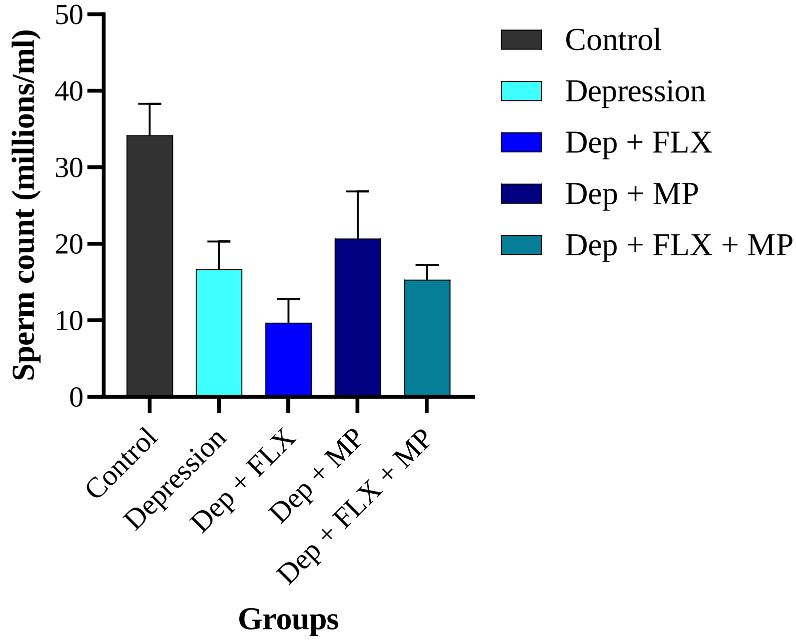


Fig no 2.3- change in sperm count in control, depression, depression+FLX, depression +MP and Depression+MP+FLX groups of rats

Sperm motility

CUMS group has shown significant decrease ($p < 0.01$) in the sperm motility in comparison to the control group & rats treated with CUMS+FLX shown significant decrease ($p<0.001$) in sperm motility when compared with control group. Rats treated with CUMS+ MP & CUMS+FLX+MP have shown a significant increase ($p < 0.01$) & ($p<0.05$) respectivelyin sperm motility compared to rats treated with CUMS+FLX. (Fig no 2.4)

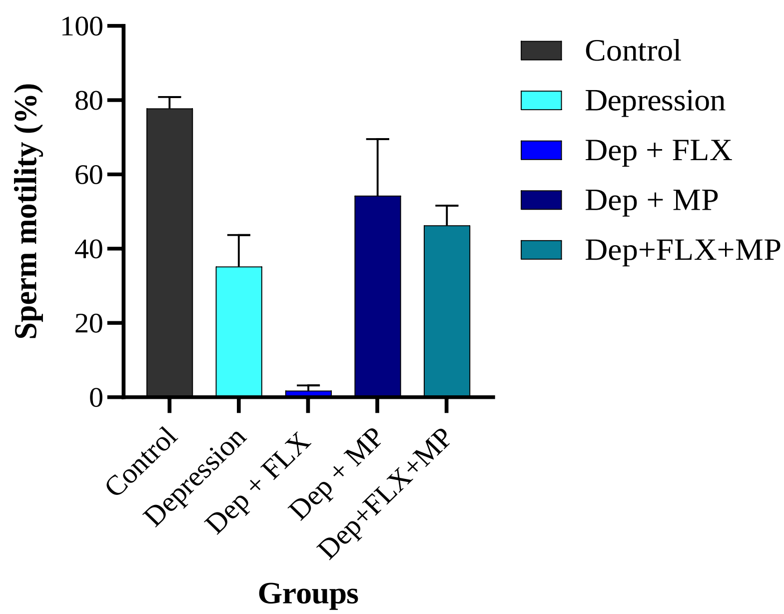
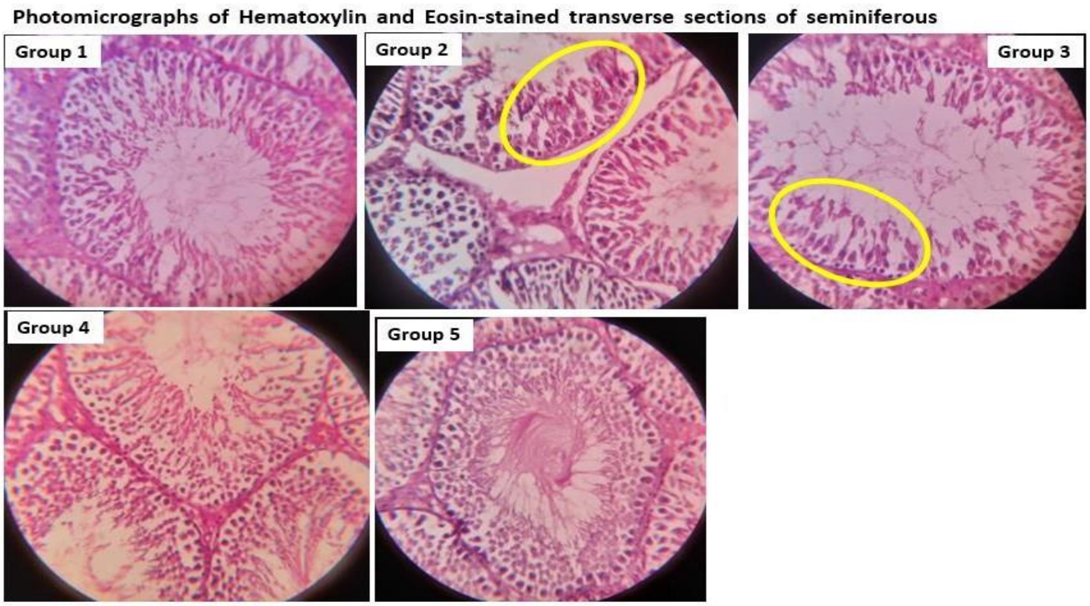


Fig no 2.4- change in sperm motility in control, depression, depression+FLX, depression +MP and Depression+MP+FLX groups of rats

Histopathological analysis

Qualitative analysis of (H&E) stained transverse sections of seminiferous tubules - H&E-stained transverse sections of seminiferous tubules of rats of control group (group 1) showed normal features of the tubules with strata of spermatogonia at different stages, thick collection of sperms in the lumen and normally looking Sertoli cells and basement membrane. Depression induced group (Group 2) and group with depression along with fluoxetine treatment (Group 3) showed depression induced damage to the seminiferous tubules such as reduced number of germ cells, seminiferous tubules without sperms and only spermatids, tubules with only Sertoli cells, and thickened basement membrane. However, Group 4 (depression along with *Mucuna pruriens* treatment) showed some recovery and features of normal seminiferous tubules though it was not at par with the control group (Group 1). But the structure of seminiferous tubules of group with depression along with *Mucuna pruriens* and fluoxetine treatment (Group 5) looked normal and close to that of control group (Group 1).(Photomicrograph 1)



Photomicrograph1 Histology of testes of control, depression, depression+FLX, depression +MP and Depression+MP+FLX groups of rats

Biochemical analysis

Testicular MDA level- Figure 4.4 CUMS group has shown significant increase ($p< 0.05$) in the testicular MDA in comparison to the control group. Rats treated with CUMS+FLX have shown a significant increase ($p< 0.001$) in testicular MDA when compared to the control group. Rats treated withCUMS+ MP & CUMS+FLX+MP showed a significant ($p<0.001$) ($p<0.01$) decrease in Testicular MDA when compared to rats treated with CUMS+FLX.(Fig no 2.5)

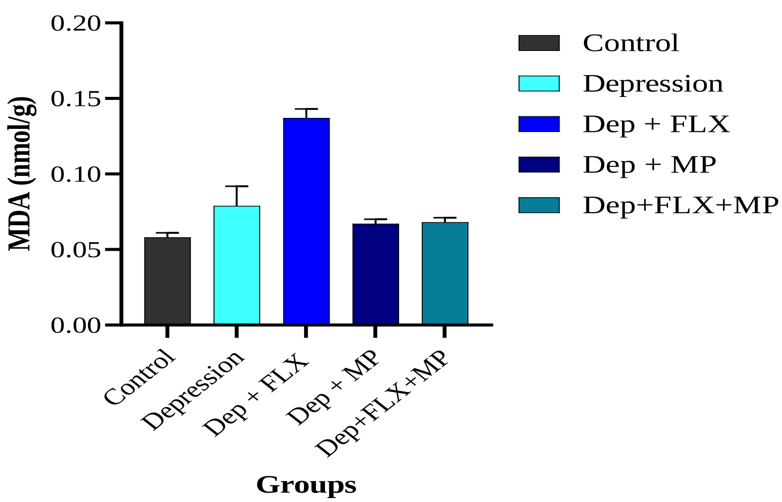


Fig no 2.5- change in MDA level in control, depression, depression+FLX, depression +MP and Depression+MP+FLX groups of rats

Testicular GSH level- Figure 4.5 CUMS group has shown significant. Decrease ($p < 0.01$) in the testicular GSH in comparison to the control group. Rats treated with CUMS +FLX have shown a significant decrease ($p < 0.001$) in testicular GSH when compared to the control. Rats treated with CUMS+MP & CUMS+FLX+MP showed a significant Increase ($p<0.05$) in Testicular MDAwhen compared to rats treated with CUMS+FLX (Fig no 2.6)

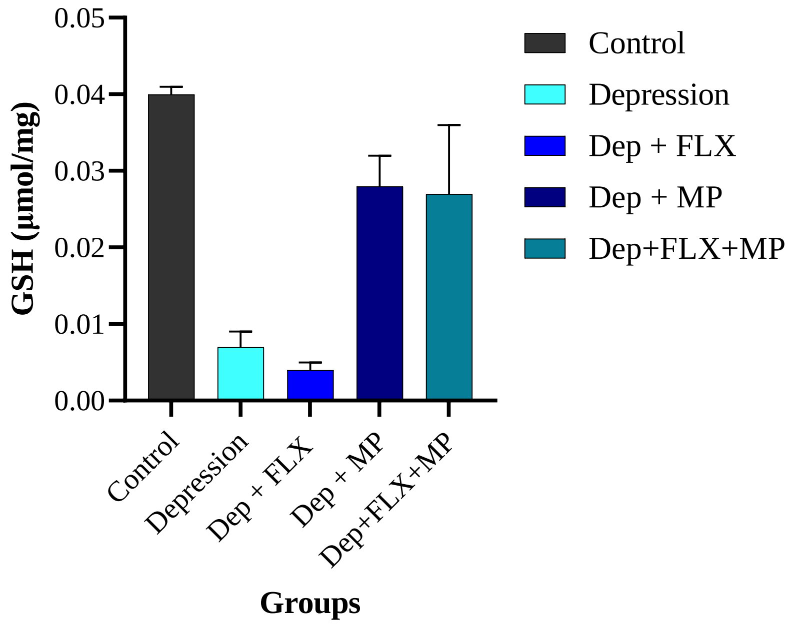


Fig no 2.6- change in GSH level in control, depression, depression+FLX, depression +MP and Depression+MP+FLX groups of rats.

DISCUSSION

In our study, overall data indicates that the protective effect of aqueous extract *Mucuna pruriens* on fluoxetine (FLX) induced gonadotoxicity in rats. In our study, there was no any significant change in the body weight of rats in all treated groups, which was similar with the research conducted by (Suresh et al 2012). Like prior research done by Bataineh and Daradka, 2007, Effects of long-term use of fluoxetine on fertility parameters in adult male rats was reported that oral administration of fluoxetine promoted decreased fertility in male albino rats. The weight of reproductive organs was markedly decreased, Significant decrease in the sperm motility of cauda epididymis was observed in the treated group. We found that rats treated with fluoxetine (FLX) had significantly reduced testis weight, along with lower sperm concentration (fig 2.3) and motility (Fig 2.4). In our investigation, aqueous extract of *Mucuna pruriens* showed a considerable improvement in testicular weight in fluoxetine (FLX) treated rats. *Mucuna pruriens* treatment successfully attenuated the effect of fluoxetine (FLX) on sperm count (fig 2.2). An earlier study reported that treatment with alcoholic extract of fumaria officinalis caused increase in, testicular weight and sperm concentration in FLX treated rats (Nasrabadi et al., 2012). Fluoxetine (FLX) treatment in rats caused a considerable raise in the percentage of nonmotile sperms it was reported by Bataineh HN, Daradka T, and Sharef et al., 2020. In our investigation also motility decreased in fluoxetine (FLX) treated rats. It was reported that there was a significant increase in sperm motility & sperm count in animals treated with sodium arsenite and Mucuna pruriens (Concessao et al., 2023). In our study also aqueous extract of *Mucuna pruriens* showed a considerable improvement in sperm motility & sperm count in Fluoxetine (FLX) treated rats. There was an increase in MDA level & decrease in GSH level in fluoxetine treated rats. This result was supported by previous study by Elsedawi, (Elsedawi et al., 2021) reported that in fluoxetine (FLX) treated rats there was increased level of MDA and decreased level of GSH in CUMS. Overproduction of MDA indicates an increase in free radical level, there was decrease in GSH level which shows decrease in natural antioxidant level. There was a decrease in MDA level & increase GSH level when aqueous extract of Mucuna pruriens is given to fluoxetine treated rats (2.5 &2.6) as *Mucuna pruriens* is rich in antioxidants could be the reason for decrease in MDA & increase in GSH levels.

In our study there was histological changes in the depression induced (group 2) and depression with antidepressant (group 3) which has shown depression induced damage to the seminiferous tubules such as reduced number of germ cells, seminiferous tubules without sperms and only spermatids, tubules with only Sertoli cells, and thickened basement membrane. But in depression + *Mucuna pruriens* (group 4) & depression+ fluoxetine + *Mucuna pruriens* (group 5) shows some recovery and features of normal seminiferous tubules. The structure of seminiferous tubules in group 5 also looks normal and at par with group (Photomicrograph1). Earlier studies found that sodium arsenite treatment caused Leydig cell degeneration, decreased sperm production and spermatid number, and a reduction in the number of epididymal sperm due to oxidative stress (Ferreira et al., 2012) It was reported that Co-administration of *Mucuna pruriens* along with sodium arsenite minimized the toxic effect of arsenic on the testis. In our study also there was some structural recovery after being treated with *Mucuna pruriens*.

The antidepressant fluoxetine induced oxidative stress, resulted in Leydig cell degeneration, reduced sperm production & spermatid quantity, & decreased epididymal sperm, Fluoxetine boosts the pituitary's production of adrenocorticotropic hormone via stimulating the hypophysial-adrenocortical axis. This causes elevated plasma corticosterone levels, which prevents gonadotropin from being produced because they make gonadotrophic cells less sensitive to the hormone-releasing gonadotropin. High levels of corticosterone & adrenocorticotropic hormone also negatively affect testosterone production & release by lowering spermatogenesis and epididymal sperm count. (Kim et al., 2015, Renu et al., 2018). Germ cells separate from the seminiferous epithelium because of decreased intratesticular testosterone levels (Juárez et L.,2017). High amounts of testosterone are necessary in the testis for healthy spermatogenesis & the preservation of the seminiferous tubule's structural integrity, which may prevent germ cell death (Walker et al., 2021, Christin et al.,2022, Heinrich et al.,2020, Zhou et al., 2019, Li et al., 2024). In our work, co-administration of *Mucuna pruriens* and fluoxetine treated rats has shown decreased toxic effects of fluoxetine on the testis reducing pituitary synthesis of adrenocorticotropic hormone & exerting its effects through the hypophysial-adrenocortical axis. From the above study we conclude the *Mucuna pruriens* treated groups depression induced or antidepressant treated rats. There was improvement in the sperm count, motility, lipid peroxidation which indicates its protective effect against fluoxetine. This beneficial effect of *Mucuna pruriens* could be due to increased endogenous level decreasing free radical level as *Mucuna pruriens* is rich in flavonoids.

CONCLUSION

In our study, *Mucuna pruriens* treatment had a positive effect on animals treated with FLX, there was an increase in sperm motility, sperm concentration and recovery changes in histopathological changes. *Mucuna pruriens* is a useful herbal remedy that may be used in conjunction with the antidepressant fluoxetine to treat patients with stress, anxiety, and depression. However, further research is required to know about the components and mechanism of *Mucuna pruriens* on gonadoprotective effect on FLX.

REFERENCES

Abarikwu S.O., Onuah C.L., Singh S.K. (2020): Plants in the management of male infertility. Andrologia. Apr 52(3):e13509. https://doi.org/10.1111/and.13509

Aruwa J.O., Bisong S.A., Obeten K., Etukudo E.My., Timothy N., Kureh T.G., Okoruwa G.A., Pius T., Usman I.M. (2024): The Potential Protective Role of Ascorbic Acid Against Testicular Toxicity Induced by Fluoxetine in Male Wistar Rats. Journal of Experimental Pharmacology. Dec 31:441-53. https://doi.org/10.2147/JEP.S476773

Bataineh H.N., Daradka T. (2007): Effects of long-term use of fluoxetine on fertility parameters in adult male rats. Neuroendocrinology letters. Jun 1;28(3):321-5.

Bataineh H.N., Daradka T. (2007): Effects of long-term use of fluoxetine on fertility parameters in adult male rats. Neuroendocrinology letters. Jun 1;28(3), 321-5.

Beeder L.A., Samplaski M.K. (2020): Effect of antidepressant medications on semen parameters and male fertility. International Journal of Urology. Jan;27(1), 39-46.doi: 10.1111/iju.14111.

Bezerra M.S., Martins A.B., Trajano F.M., de Araujo Pontes T.H., da Costa Gomes L.T., Gavioli E.C., da Silva Junior E.D. (2019): Fluoxetine and sertraline effects on rat distal cauda epididymis contraction, sperm count and sperm transit time trough epididymis. European Journal of Pharmacology. Dec 15, 865:172774. https://doi.org/10.1016/j.ejphar.2019.172774

Christin-Maitre S., Young J. (2022): Androgens and spermatogenesis. InAnnales d'endocrinologie. Jun 1, 83(3), 155-158. Elsevier Masson. https://doi.org/10.1016/j.ando.2022.04.010

Concessao P. L., Bairy, K. L., & Raghavendra, A. P. (2023): Ameliorating effect of Mucuna pruriens seed extract on sodium arsenite-induced testicular toxicity and hepato-renal histopathology in rats. Veterinary world, 16(1), 82–93. https://doi.org/10.14202/vetworld.2023.82-93

Elsedawi B.F., Hussein Y., Sabry M.A., Aziz J.A. (2021): Effect of fluoxetine on the testes of adult albino rats and the possible protective role of curcumin. Anatomical Science International. Mar; 96,187-96. https://doi.org/10.1007/s12565-020-00573-9

Ferreira M., Matos R.C., Oliveira H., Nunes B., Pereira M.D. (2012): Impairment of mice spermatogenesis by sodium arsenite. Human & experimental toxicology. Mar;31(3), 290-302. https://doi.org/10.1177/0960327111405862

Gocmez S.S., Utkan T., Ulak G., Gacar N., Erden F. (2010): Goods of long- term treatment with fluoxetine and venlafaxine on rat insulated vas deferens. Auton Autacoid Pharmacol; 30, 197-202 https://doi.org/10.1111/j.1474-8673.2010.00456.x

He L.W., Zeng L., Tian N., Li Y., He T., Tan D.M., Zhang Q., Tan Y. (2020): Optimization of food deprivation and sucrose preference test in SD rat model undergoing chronic unpredictable mild stress. Animal models and experimental medicine. Mar;3(1), 69-78. https://doi.org/10.1002/ame2.12107

Heinrich A., DeFalco T. (2020): Essential roles of interstitial cells in testicular development and function. Andrology. Jul;8(4), 903-14. https://doi.org/10.1111/andr.12703

Juárez-Rojas L., Vigueras-Villaseñor R.M., Casillas F., Retana-Márquez S. (2017): Gradual decrease in spermatogenesis caused by chronic stress. Acta Histochemica. Apr 1;119(3), 284-91. https://doi.org/10.1016/j.acthis.2017.02.004

Kerr J.B., Maddocks S., Sharpe R.M. (1992): Testosterone and FSH have independent, synergistic and stage-dependent effects upon spermatogenesis in the rat testis. Cell and tissue research. Apr;268, 179-89. https://doi.org/10.1007/BF00338067

Khan M.Y., Kumar V. (2017): Medium of antihypertensive effect of Mucuna pruriensL..seed extract and its isolated compounds. J Compl Integr Med.;14(4): 1e11. https://doi.org/10.1515/jcim-2017-0014

Kim Y.J., Kim J.M. (2015): Arsenic toxicity in male reproduction and development. Development & reproduction. Dec;19(4), 167. https://doi.org/10.12717/DR.2015.19.4.167

Lampariello L.R., Cortelazzo A., Guerranti R., Sticozzi C., Valacchi G. (2012): The magic velvet bean of. Mucuna pruriens. J Tradit Complement Med.2;2(4):331e339. https://doi.org/10.1016/S2225-4110(16)30119-5

Lapyuneyong N., Tangsrisakda N., Choowong-In P., Chaisiwamongkol K., Uabundit N., Sawatpanich T., Arun S., Wu A.T., Iamsaard S. (2022): Seed extract of Thai Mucuna pruriens reduced male reproductive damage in rats induced by chronic stress. Pharmaceutical Biology. Dec 31;60(1), 374-83. https://doi.org/10.1080/13880209.2022.2034896

Li L., Lin W., Wang Z., Huang R., Xia H., Li Z., Deng J., Ye T., Huang Y., Yang Y. (2024): Hormone Regulation in Testicular Development and Function. International Journal of Molecular Sciences. May 26;25(11), 5805. https://doi.org/10.3390/ijms25115805

Lynda E.O., Kingsley N.E., Obukohwo O.M., Benneth B.A., Victor E., Simon O.I., Agbonifo-Chijiokwu E., Oghenetega O.B. (2024): Arjunolic acid reverses fluoxetine-induced alterations in testicular steroidogenic enzymes and membrane bound ionic pump imbalance through suppression of oxido-inflammatory stress and apoptosis. JBRA Assisted Reproduction. Jan;28(1), 66. https://doi.org/10.5935/1518-0557.20230062

Marwaha S., Palmer E., Suppes T., Cons E., Young A.H., Upthegrove R. (2023): Novel and emerging treatments for major depression. The Lancet. Jan 14;401(10371):141-53. https://doi.org/10.1016/S0140-6736(22)02080-3

Monteiro Filho W.O., Torres S.M., Amorim M.J., Andrade A.J., Morais R.N., Tenorio B.M., Silva Junior V.A. (2014): Fluoxetine induces changes in the testicle and testosterone in adult male rats exposed via placenta and lactation. Systems Biology in Reproductive Medicine. 2014 Oct 1;60(5):274-81. https://doi.org/10.3109/19396368.2014.933984

Nasrabadi M.H., Aboutalebi H., Naseri M. (2012): Effect of Fumaria parviflora alcoholic extract on male rat's reproductive system. J Med Plants Res; 6(10):2004–10. 23. Aggarwal A, Jethani S L, Rohatgi R K

Nayanatara A.K., Nagaraja H.S., Anupama B.K. (2005): The effect of repeated swimming stress on organ weights and lipid peroxidation in rats. Thai J Pharm Sci. 2005 Apr;18(1):3-9.

Porsolt R., Le Pichon M. & Jalfre M. (1977): Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**, 730–732. https://doi.org/10.1038/266730a0

Rekha D.K., Nayanatara A.K., Ramaswamy, Sheila R.P., Ramesh Bhat, Venkappa S.M. (2009): Infertility in male wistar rats induced by cadmium chloride: role of ascorbidacid. Journal of Chinese clinical medicine. 4(11), 616-21.

Renu K., Madhyastha H., Madhyastha R., Maruyama M., Vinayagam S., Gopalakrishnan A.V. (2018): Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity. Life sciences. Nov 1;212:37-58. https://doi.org/10.1016/j.lfs.2018.09.045

Sharef A.Y., Aziz F.M., Adham A.N. (2020): The protective effect of Fumaria officinalis against the testicular toxicity of fluoxetine in rat. Zanco Journal of Medical Sciences (Zanco J Med Sci. Apr 30;24(1):117-31. https://doi.org/10.15218/zjms.2020.015

Shukla K.K., Mahdi A.A., Ahmad M.K., Jaiswar S.P., Shankwar S.N., Tiwari S.C. (2010): Mucuna pruriens reduces stress and improves the quality of semen in infertile men. Evidence-Based Complementary and Alternative Medicine.7(1):137-44. https://doi.org/10.1093/ecam/nem171

Sinha S., Sharma S., Vora J., Shah H., Srivastava A., Shrivastava N. (2018): Mucuna pruriens (L.) DC chemo sensitizes human breast cancer cells via downregulation of prolactin-mediated JAK2/STAT5A signaling. J Ethnopharmacol. 217, 23e35. https://doi.org/10.1016/j.jep.2018.02.006

Suresh S., Prakash S. (2012): Effect of Mucuna pruriens (Linn.) on sexual behavior and sperm parameters in streptozotocin-induced diabetic male rat. The journal of sexual medicine. Dec;9(12), 3066-78. https://doi.org/10.1111/j.1743-6109.2010.01831.x

Suresh S., Prithiviraj E., Lakshmi N.V., Ganesh M.K., Ganesh L., Prakash S. (2013): Effect of Mucuna pruriens (Linn.) on mitochondrial dysfunction and DNA damage in epididymal sperm of streptozotocin induced diabetic rat. Journal of Ethnopharmacology. Jan 9;145(1):32-41. https://doi.org/10.1016/j.jep.2012.10.030

Walker W.H.(2021): Androgen actions in the testis and the regulation of spermatogenesis. Molecular Mechanisms in Spermatogenesis. 175-203. https://doi.org/10.1007/978-3-030-77779-1_9

Yao Y., Ju P., Liu H. et al.(2020*)*: Ifenprodil rapidly ameliorates depressive-like behaviors, activates mTOR signaling and modulates proinflammatory cytokines in the hippocampus of CUMS rats. *Psychopharmacology.* **237**, 1421–1433. https://doi.org/10.1007/s00213-020-05469-0

Zhou R., Wu J., Liu B., Jiang Y., Chen W., Li J., He Q., He Z.(2019): The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. Cellular and molecular life sciences. Jul 30;76:2681-95. https://doi.org/10.1007/s00018-019-03101-9