ORIGINAL ARTICLE

Moringa Oleifera Protects Against Fluoxetine Induced Damage to the Basement Membrane of the Seminiferous Tubules in Adult Male Rats

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ABSTRACT

Objective: To explore the protective effects of fluoxetine and *moringa oleifera* on the basement membrane of seminiferous tubules in the adult male rat testis.

Study Design: Randomized Experimental Study.

Place and Duration of Study: The study was conducted at the Anatomy Department of Army Medical College, National University of Medical Sciences (NUMS) Rawalpindi, in collaboration with the National Institute of Health Sciences (NIH) Islamabad and Pak Emirates Military Hospital (PEMH) Rawalpindi, from 1st June 2022 to 1st May 2023.

Materials and Methods: Thirty male Sprague Dawley rats weighing 300 ± 50 grams with no obvious gross abnormality were randomly divided into three groups (n=10). Daily doses were administered via oral gavage for 8 weeks. The group A (control) received distilled water. The group B (experimental) received fluoxetine at a dose of 10 mg/kg/day, and the group C (experimental) was given *moringa oleifera* powder at a dose of 50 mg/30 g body weight. The rats were sacrificed and the disruption of the basement membrane of seminiferous tubules was assessed using a scoring system (0 to 3). The significance was calculated using cross tabs by applying Chi-Square test using SPSS version 22. The p value ≤ 0.05 was considered statistically significant.

Results: Group A rats had no disruption of basement membrane. In group B, 50% of specimens exhibited severe disruption, 40% had moderate disruption, and 10% had slight disruption. In group C, 10% of specimens showed moderate disruption, 30% showed slight disruption, and 60% had no disruption. A statistically significant difference was observed between groups B and C ($p < 0.05$).

Conclusion: Fluoxetine significantly disrupts the basement membrane of seminiferous tubules, adversely affecting spermatogenesis. Conversely, *moringa oleifera* demonstrates a protective effect against such disruptions indicating its potential therapeutic use.

Key Words: *Basement membrane, Fluoxetine, Moringa oleifera, Spermatogenesis, Testis.*

Introduction

Depression is a mood disorder that affects the physical and psychological aspects of a person.[1] It causes a persistent feeling of sadness and loss of interest in daily routine activities that were previously enjoyable.[2] Like other chronic diseases, depression can be debilitating however, it is often ignored and stigmatized.[3] Selective serotonin reuptake inhibitors (SSRIs) are the antidepressants most frequently prescribed to treat depression worldwide.[1] Despite its miraculous effects on relieving anxiety and depression, it also interferes with hypothalamic pituitary gonadal pathway (HPG), blocking dopamine receptors and increasing prolactin levels.[4] This in turn inhibits gonadotropin releasing hormone (GnRH) from hypothalamus, causing ultimately a decrease in testosterone levels leading to sexual dysfunction and affecting the process of spermatogenesis.[5] It also induces local testicular injury in both the interstitial and tubular compartments of testis which leads to infertility in males.[6] So, prevention of testicular toxicity has been considered an important strategy to restore fertility. Fluoxetine causes increase in the level of malondialdehyde (MDA) and lowers the level of superoxide dismutase (SOD) in testis and there is also

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evidence that it is protective against oxidative stress.[7] This redox condition is mostly caused by mitochondrial malfunction. The inhibition of the electron transport chain in damaged mitochondria results in the production of reactive oxygen species (ROS), depletion of energy, consumption of antioxidants, accumulation of cytotoxic mediators, and eventual cell death.[8]

Plants have been in use all over the world for fertility regulating characteristics. Some medicinal plants are widely used as aphrodisiacs to alleviate sexual dysfunction or as fertility boosting agents.[9] *Moringa oleifera*, locally known as "Lam" has been known as the "miracle tree" since ages.[10] It has got incredible nutritional and health benefits. Being rich in multiple macro and micronutrients it serves to cure a lot of diseases. Different parts of the plant such as leaves, flowers and seeds have potent therapeutic effects and are used as antidiabetic, anticancer, antiulcer, antimicrobial and antioxidant.[11] The plant is commonly found in India, Pakistan, Afghanistan, and Bangladesh. It is the drought resistant species of a mono generic family, the *Moringaceae.*[12] Antioxidant property of *moringa oleifera* is because of its specific constituents like flavonoids, carotenoids, phenol and vitamin A.[13] Carotenoids not only act as antioxidants but also protect against aging and cellular damage. Vitamin A content of *moringa* is important in regulating vision and reproduction.[14]

Phenolic and flavonoids compounds like gallic acid, chlorogenic acid and vanillin are also rich in *moringa oleifera*. Phenolics and flavonoids have been described to have a powerful antioxidant property and high ability to reduce protein oxidation and DNA damage leading to the inhibition of cellular injury.[10] There is little literature to support free radical hunting ability of *moringa oleifera* in terms of testicular toxicity. So, this study focused on the antioxidant property of *moringa oleifera* in ameliorating the testicular toxicity induced by fluoxetine in testis of adult male rats.

Materials and Methods

It was a randomized experimental study, (ERC/ID/216). The study was conducted at the anatomy department of Army Medical College, National University of Medical Sciences NUMS Rawalpindi in collaboration with the National Institute of Health Sciences NIH Islamabad and Pak Emirates Military Hospital PEMH Rawalpindi. The duration of the study was from 1st June 2022 to 1st May 2023. The rules and regulations regarding the handling and care of animals were strictly followed and set forth by the Ethics Review Committee of Army Medical College. Thirty male Sprague Dawley rats 3-4 months of age, having an average weight of 300 ± 50 grams were obtained from NIH Islamabad. Rats were randomly divided into three groups with 10 rats each and 5 rats housed in one cage. Rats were kept under the standard lab conditions of a daily photoperiod of a 12hr dark-light cycle. Rats were allowed free access to the standard lab diet and clean drinking water *ad libitum* for 08 weeks.

Fluoxetine capsules (20mg) were purchased from the local market, and powder was dissolved thoroughly in distilled water to make a 2% w/v solution of the extract. *Moringa oleifera* was also used in its finely grounded powdered form in sealed packages from the Pakistan Agricultural and Research Council PARC Islamabad. The powdered herb was dissolved in distilled water to make a 2% w/v solution, which was sieved to get an extract. The extract was used in the study.

The drug and herb were given in a single daily dose through oral gavage. The group A was kept as control and 5ml of distilled water was given. The groups B and C were the experimental groups. The group B was given fluoxetine 10mg/kg body weight dissolved in distilled water.[15] The group C was given both the drug and the herb, fluoxetine in the same daily dose as was given in group B and *moringa oleifera* in a dose of 50mg/30 grams body weight dissolved in distilled water.[12]

The rats were euthanized in transparent glass chambers with cotton soaked in diethyl ether. The sacrifice was done after the end of the experimental period 24 hours after the administration of the last dose. The right testis was selected for histomorphometry as a standard. The testes were placed in 10% formalin. The tissue processing was done in an ascending order of ethyl alcohol followed by processing in Leica TP 1020 tissue processor. Hematoxylin and Eosin H&E stains were used for staining. The histomorphometric analysis of histological sections was performed using a light microscope with a 10X eyepiece and a 10X objective lens, providing a total magnification of 100X.

The disruption of the basement membrane was analyzed using a scoring system (0 to 3). To maintain uniformity, tubules were counted moving from right to left in equally spaced consecutive fields. The disruption of basement membrane was then analyzed in each selected seminiferous tubule and scored from 0 to 3.[16] According to scale, 0= no disruption, 1=slight disruption (≤ 50% of the tubule cross section shows disruption, 2= moderate disruption (≥ 50% of the tubule cross section shows disruption, 3= severe disruption (≥ 70% of the tubule cross section shows disruption).[16]

Statistical Package for the Social Sciences version 22 (SPSS V.22.0) was used to analyze the data. The significance was calculated using cross tabs by applying Chi-Square test. The *p* value ≤ 0.05 was considered statistically significant.

Results

Group A rats had no disruption of basement membrane (Figure-I). In group B, 50% of specimens exhibited severe disruption, 40% had moderate disruption, and 10% had slight disruption (Figure-II). In group C, 10% of specimens showed moderate disruption, 30% showed slight disruption, and 60% had no disruption (Figure-III). A statistically significant difference was observed between groups B and C *(p < 0.05)*. (Table-I & Table-II).

Table I: Mean Values of The Disruption of The Basement Membrane of the Seminiferous Tubule of the three Experimental Groups (n=10).

	Scoring	Group A	Group B	Group C
Disruption of Basement Membrane	0 = No effect	10 (100%)	0 (0%)	6 (60%)
	1= Mild Disruption	0 (0%)	1 (1%)	3 (30%)
	2 = Moderate Disruption	0 (0%)	4 (40%)	1 (10%)
	3 = Severe Disruption	0 (0%)	5 (50%)	0 (0%)

Table II: Disruption of Basement Membrane Comparison Between Groups (n=10).

	Group A vs B	Group A vs C	Group B vs C
Disruption of basement membrane	<0.001	0.087	<0.001

Discussion

This study showed that fluoxetine, which is a commonly prescribed SSRI causes significant

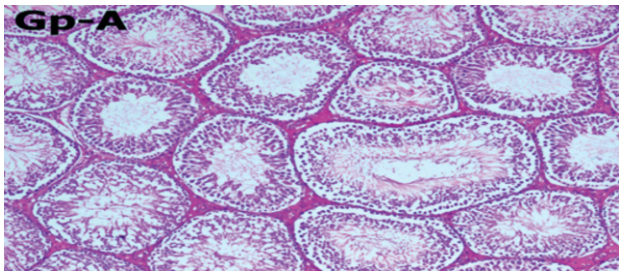

Figure 1: A Photomicrograph of Group A Showing no Disruption of the Basement Membrane of the Seminiferous Tubules (100X, H&E).

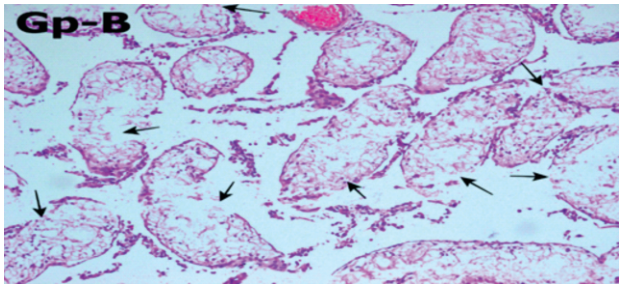

Figure 2: A Photomicrograph of Group B Showing Disruption of the Seminiferous Tubules (100X, H&E)

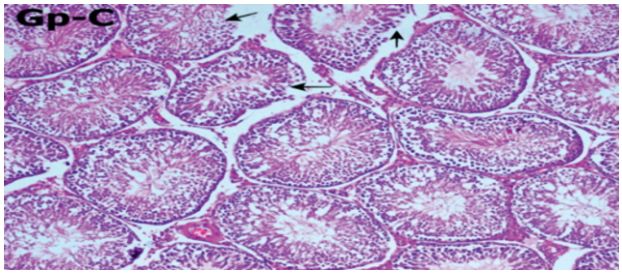

Figure 3 : A photomicrograph of Experimental Group C Showing Less Disrupted Seminiferous Tubules (f100X, H&E)

disruption in the basement membrane of the seminiferous tubules in the testes of male rats. In our study, the control group showed no distortion whereas the experimental group B treated with fluoxetine showed a higher percentage of severely distorted basement membranes followed by moderate distortion.

The current findings are in accordance with the study done by Asad A *et al*.,[16] where toxic effects of lead-induced oxidative stress and effected the basal lamina of the seminiferous tubules but in present study toxicity was induced by fluoxetine. Another study in line with the present study was done by Johnson *et al*.,[17] where chemical injury led to disruption of the basal lamina. Fluoxetine induces an imbalance between the prooxidants and antioxidants in the body which leads to production of ROS that causes lipid peroxidation and damages the

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susceptible membrane of seminiferous tubules rich in polyunsaturated fatty acids (PUFA).[18] Experimental group C treated concomitantly with *moringa oleifera* showed 10% moderate and 30% mild distortion. While 60% of tubules showed no distortion at all. This explained its occurrence due to the presence of naturally occurring antioxidants present in *moringa oleifera* like flavonoids, phenols, antioxidative vitamins, and antioxidative enzymes like quercetin in it.[19] A study conducted by Abd HH *et al.,*[20] also goes with the present study where antioxidants in *moringa oleifera* modulated oxidative stress and thus prevented testicular injury. Another study conducted by Naheed *et al.,*[21] goes with our study where presence of flavonoids and phenols in the medicinal herb protected the basement membrane of the seminiferous tubules from damage but in this study toxicity was induced by microwave radiations.

Study conducted by Opuwari *et al.,*[22] also credited antioxidant potential of *moringa oleifera* being responsible for enhancing testis defense against oxidative assault caused by reactive oxygen species (ROS) but the study was conducted on Wistar rats.

Another study conducted by Mohlala *et al*.,[23] also attributed oxidative stress in testis responsible for causing DNA damage, lipid peroxidation and protein oxidation in reproductive cells, whereas *moringa oleifera* owing to its antioxidant potential being rich in vitamin B, C, beta carotene, ferulic acid, gallic acid improves the histological parameters of the testis.

Our study is also supported by the research done by Habib *et al*.,[24] where vitamin E was found to have a potent protective effect on the basement membrane of the seminiferous tubules but the toxicity was induced by phthalate. Moringa has a rich concentration of vitamin E in it and serves to guard the integrity of the basement membrane of the seminiferous tubules.

Ayse Busra *et al*.,[25] also conducted a study showing the potent nature of antioxidant vitamin E responsible for raising the levels of endogenous antioxidants SOD (superoxide dismutase) and lowering the levels of MDA (malondialdehyde) which had a protective effect on testicular histology. But the study differs from ours as we made use of moringa herb that is rich in antioxidative vitamins C and E, but the above-mentioned study directly used vitamin E as an ameliorative agent.

This study has specifically focused on structural disruption of the basement membrane of the seminiferous tubules because of fluoxetine induced toxicity using a standardized scoring system, an area reported in very few studies. Also, the comparative evaluation of fluoxetine and *moringa oleifera* provides new insight into the protective role of *moringa oleifera* demonstrating its efficacy in mitigating the damage to basement membrane. This indicates its potential therapeutic use as a natural antioxidant for preventing drug induced testicular damage. These findings pave the way for future research into therapeutic strategies for reproductive toxicity.

Limitations and Recommendations

The study period should be longer to observe the toxic effects of fluoxetine and ameliorative effects of moringa herb. Biochemical analysis along with antioxidant markers can be added to value the outcome.

Conclusion

The study showed that fluoxetine has toxic effects on the testis of adult male rats by disrupting spermatogenesis showed by disruption of the basement membrane of the seminiferous tubules. *Moringa oleifera's* antioxidative properties counteract fluoxetine-induced testicular toxicity indicating its potential therapeutic use.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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