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Ameliorative Effects of Moringa Oleifera on Histomorphology of Rat Testis Treated with Fluoxetine

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ABSTRACT

Objective: To observe the histomorphological changes in rat testes treated with Fluoxetine, focusing on the weight of the testis along with the epithelial thickness of the seminiferous tubules and evaluating the ameliorative effects of Moringa oleifera on the same gross and histological parameters.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Army Medical College, National University of Medical Sciences (NUMS), with the National Institute of Health, Islamabad and Pak Emirates Military Hospital, Rawalpindi Pakistan, from Sep 2022 to Mar 2023.

Methodology: Thirty male Sprague Dawley rats divided into three groups (n=10). Group-A was the Control-Group, and the rest served as the Experimental-Group. Drugs were given in a single daily dose through oral gavage for eight weeks. Group-B was given Fluoxetine, 10 mg/kg body weight. Group-C was administered Fluoxetine in the same dose and concomitant administration of Moringa powder, 50mg/30 grams body weight. After sacrifice, testes were fixed and stained with Hematoxylin and Eosin for histological study.

Results: The study found a statistically significant decrease in the weight of rat testis (p-value<0.05) and epithelial thickness of seminiferous tubules of rats treated with Fluoxetine (p-value <0.05).

Conclusion: Fluoxetine significantly decreased the weight of the testis and epithelial thickness of the seminiferous tubules in rat testes. Moringa Oleifera administration ameliorated these effects in the short term.

Keywords: Antidepressants, Fluoxetine, Moringa oleifera, SSRIs, Seminiferous tubules, Testicular toxicity.

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INTRODUCTION

Depression and anxiety disorders pose serious threats to the mental health of society worldwide.[1] The World Health Organization (WHO) declared depression to be the second largest burden of ischemic heart disease. It affects an individual's appearance, thinking, mental, social, and economic status.[2]

Selective Serotonin Reuptake Inhibitors (SSRIs) are the most frequently used antidepressants. Several members of this group include Fluoxetine, sertraline, paroxetine, dapoxetine, citalopram, & escitalopram.[3,4] Among these, Fluoxetine is considered to be the most potent. Despite its miraculous effects in treating depression, it also has untoward effects on human health. Fluoxetine induces oxidative stress in testicular tissue, ultimately affecting its function.[5] Free radicals formed by oxidative stress in the testes disrupt spermatogenesis, which curtails patient fertility.[6]

The basic need for food and curiosity about nature have led to the discovery of more than a million plants that have been classified and domesticated. One such small plant is an herb native to Southeast Asia (Moringa Oleifera), also known as the drumstick and horseradish trees. It contains amino acids and carotenoids, making it useful for the skin, nails, bones, and wound healing.[7] Moringa Oleifera has antioxidant properties because of its specific constituents such as flavonoids, carotenoids, phenol, and Vitamin A. Phenolic and flavonoid compounds such as gallic acid, chlorogenic acid, and vanillin are also abundant in Moringa Oleifera, which have powerful antioxidant properties, free radical hunting ability, and high ability to reduce protein oxidation and DNA damage, leading to the inhibition of cellular injury.[8] Many studies have shown the injurious effects of Fluoxetine on the testis, but its amelioration with Moringa Oleifera has not been previously reported.[9,10] Therefore, the rationale of this study was to determine the role of Moringa Oleifera in ameliorating testicular toxicity in male Sprague Dawley rats induced by Fluoxetine.

METHODOLOGY

The laboratory-based experimental study was conducted at the Department of Anatomy, Army Medical College, National University of Health Sciences (NUMS) in collaboration with the National Institute of Health (NIH), Islamabad and Pathology Laboratory of

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Pak Emirates Military Hospital (PEMH), Rawalpindi Pakistan from September 2022 to March 2023.

Inclusion Criteria: All male Sprague Dawley rats aged 3-4 months with an average weight of 350±50 grams were included.

Exclusion Criteria: Male rats with any gross abnormalities were excluded from the study.

Thirty male Sprague-Dawley rats, were purchased from NIH. Non-probability convenience sampling was performed, and the rats were randomly divided into three groups, with ten rats in each group and five rats per cage. The rats were kept in the animal house of the NIH, and standard laboratory conditions were ensured. Access to a standard lab diet and clean drinking water were provided ad libitum. Rats were kept in a well-aerated room where a temperature of 22–24°C was maintained throughout the experimental period. A daily photoperiod of approximately 12 h dark-light sleep cycle was ensured for the duration of the experiment. All drugs were administered in a single daily dose and dissolved in distilled water by oral gavage for eight weeks.

In sealed packaging, the herb was purchased in soft, finely ground powder form from the Pakistan Agricultural Research Council, Islamabad Pakistan. Finely ground powder was used in this study so that it could be easily dissolved in distilled water. The solution was filtered, and the extract was used in the study.

Fluoxetine capsules were purchased from a local market, and finely ground powder was dissolved in distilled water. Group-A was the Control-Group, so no drugs or herbs were administered. The rats were administered 5 ml of distilled water by oral gavage. Groups B and C were Experimental Groups. The animals in Group B were administered fluoxetine 10 mg/kg body weight 7. In Group C, Moringa Oleifera 50mg/30 g body weight 8 was concomitantly administered in addition to the same dose of Fluoxetine administered to Group-B. All three groups were observed for eight weeks.

At the end of the experimental period, rats were euthanised with an overdose of diethyl ether. After euthanasia, the rats were sacrificed, and the right testis was dissected to keep it as a standard. The weight of each right testis was calculated in grams (g). The selected testes were placed in 10% Formalin solution for preservation in labelled plastic containers. Sectioning was performed at the histopathology laboratory of PEMH in Rawalpindi Pakistan. Cross sections were obtained from two different levels of each testis, one from the upper and the other from the lower. The tissues were then placed in appropriately labelled cassettes. Processing was performed using an automatic tissue processor Leica TP 1020 and then embedded in melted paraffin wax to make blocks. Sections of 5µm thickness were obtained using a rotary microtome, and the slides were stained with Hematoxylin and eosin (H&E). Microscopy was performed using a light microscope at 40X magnification. The mean epithelial thickness (µm) was recorded using a micrometre.

Two round cross-sections of the seminiferous tubules were randomly selected from three consecutive fields at 40X. Thickness was obtained by measuring the distance from the basement membrane to the top layer of the luminal surface from four sides (four angles) using micrometry. The calculation was completed by calculating the average of four readings. The mean of all readings was considered the final reading for that specimen. The weight of the right testis of each animal was recorded in grams using an electronic analytical and precision balance (BA 210S, d=0.001-Sartoriusen GA, Germany).

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. One-way analysis of variance (ANOVA) was applied to gauge the mean differences among the groups. The group differences were calculated using Post Hoc test (Tukey HSD). The *p*-value of 0.05 or less was taken as significant.

RESULTS

Thirty male Sprague Dawley rats 3-4 months of age, having an average weight of 300±50 grams without any gross abnormality, were included. The effect of Fluoxetine and ameliorative effects of Moringa herb were observed on the testicular weight of the rats and epithelial thickness of the seminiferous tubules. After dissection, the weight of the testes was observed and was found to be statistically significant *p*-value <0.001 when Group-B, with a mean value of 1.91±0.138, was juxtaposed to Group-C, with a mean value of 2.33±0.204. The statistically significant result of *p*-value<0.001 was found when Group-A, with a mean value of 2.69±0.139, was compared to Groups B and C was compared to (Table-I).

Table-I: Comparison of the Mean Weight of the Testis and Epithelial Thickness of the Seminiferous Tubules between the three study groups (n=30)

Parameters	Group-A (n=10) Mean ±SD	Group-B (n=10) Mean ±SD	Group-C (n=10) Mean±SD	*p*-value
Weight of the testis (gm)	2.69±0.139	1.91±0.138	2.33±0.204	<0.001
Epithelial Thickness of the Seminiferous Tubules (µm)	82.79±4.39	53.29±4.56	79.38±4.02	<0.001

Regarding epithelial thickness, the Mean±SD was found to be 53.29±4.56 in Group-B, which was a statistically significant *p*-value<0.001 to Group-C, having a mean value of 79.38±4.02. An insignificant result (*p*-value >0.05) was found when Group-A, with a mean value of 82.79±4.39, was compared to Group-C. Inter-group comparison (Post Hoc analysis) of the weight of the testis and Epithelial Thickness of the Seminiferous Tubules between the three study groups is shown in the Table-II. Figure shows photomicrographs showing the epithelial thickness of the seminiferous tubules in Control Group-A and Experimental Groups B and C.

Table II: Inter-group comparison table (Post Hoc analysis) of the weight of the testis and Epithelial Thickness of the Seminiferous Tubules between the three study groups (n=30)

Parameters	Group-A Vs. Group-B	Group-B Vs. Group-C	Group-A Vs. Group-C
Weight of the testis (gm)	<0.001	<0.001	<0.001
Epithelial Thickness of the seminiferous tubules (µm)	<0.001	<0.001	0.202

DISCUSSION

Moringa Oleifera is an amazing discovery as it has multidimensional effects coming to light over time. In our study, we used Fluoxetine, which has been used for the treatment of neurological illnesses for quite a long time but has known adverse effects on the male reproductive system. Therefore, we paired both chemicals to determine whether Moringa Oleifera ameliorates fluoxetine-induced testicular toxicity. The weight of the accessory sex organs and testes is a reliable index for measuring the toxic effect of a drug, particularly SSRIs.[11] The testis appeared smooth, soft, pink, and spongy on gross examination. In this study, the weight of the testis decreased in the fluoxetine-treated group. This finding is supported by a study conducted by Camara *et al*. which explained that considerable dissociation and loss of germ cells resulted in reduced tubular and epithelial areas, leading to reduced testis weight.[12] A study demonstrating a reduction in testicular weight in response to Fluoxetine was also conducted by El Roghy *et al*.[13] However, the difference from this study lies in the selection of albino rats. Another similar study in support of this study was conducted by Elsedawi *et al*.[14] who claimed that Fluoxetine can reduce LH release from the anterior pituitary lobe and suppress steroidogenesis by preventing serotonin reuptake. Adult albino rats were chosen for this study.

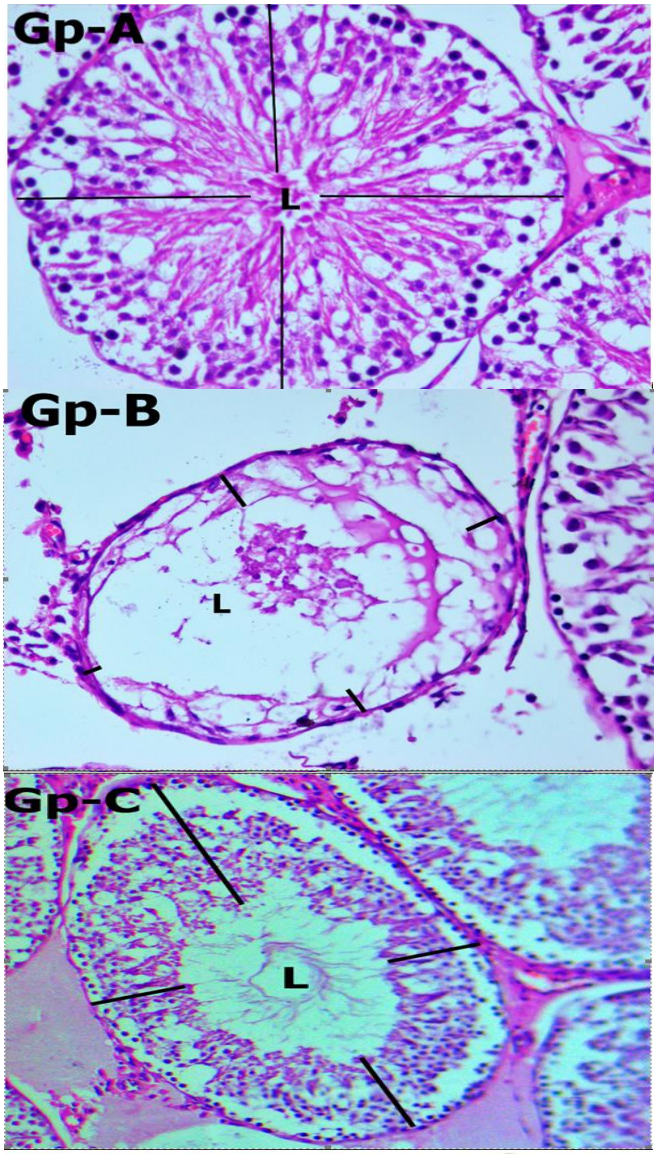

Figure: Photomicrographs showing the epithelial thickness of the seminiferous tubules in control group A and experimental groups B and C where lines = epithelial thickness, L= lumen.

Krimipour *et al*. also demonstrates the toxicity of Fluoxetine suppressed steroidogenesis, which ultimately reduced the weight of the testis in rat pups [15]. An increase in the weight of the testis was observed in Experimental Group-C, which was administered Moringa Oleifera along with the same dose of Fluoxetine. This increase was statistically significant in comparison to Group-B. This is consistent with one study who observed the same effects of Moringa oleifera on the testes of male mice. Obeme and Raji used Moringa oleifera seed extract. They observed a similar increase in testicular weight in male Wistar rats, while Sprague Dawley rats were used in our study.[16] However, tests of different rats show few morphological differences. Mostafa assumed zinc in Moringa was responsible for increasing the weight of the testis in adult male rats. Zinc acts as an antioxidant that plays an integral role in protecting the sperm membrane, thereby making it more motile and less vulnerable to damage by oxidative stress.[17]

The outcome of this study was in accordance with the inference drawn from a study conducted by Elsedawi *et al*.[14] in which Fluoxetine induced testicular damage in adult albino rats. This can be explained as Fluoxetine limiting LH release from the anterior pituitary lobe and suppressing steroidogenesis by preventing serotonin reuptake. However, this effect was observed in Sprague Dawley rats in the present study. Another study in favour of our study was conducted by El-Roghy *et al*.[13] which demonstrated the injurious effects of Fluoxetine on seminiferous tubules, causing membrane disruption and shedding of germ cells, reducing the height of the germinal epithelium; however, effects were observed in adult albino rats. In Group C, improvement in the epithelial thickness of the seminiferous tubules was observed because of the concomitant administration of Moringa Oleifera with Fluoxetine. This is supported by a study conducted by Ramalingam *et al*.[18] which showed that certain micronutrients in Moringa Oleifera have enhanced antioxidant properties that help prevent tissue damage from free radicals.

LIMITATION OF THE STUDY

Increasing the sample size and the duration of the study period will produce more accurate results. Biochemical analysis and immunohistochemistry should be paired with histological parameters, but this poses some financial restraints for the study.

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CONCLUSION

The study established that Fluoxetine significantly reduced testicular weight and epithelial thickness of the seminiferous tubules. At the same time, Moringa Oleifera significantly ameliorated both gross and histological changes in terms of an increase in the weight of the testis and epithelial thickness of the seminiferous tubules of Sprague Dawley rats.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AA & AQ: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

SH & TF: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

TK & FM: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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