ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 14, Issue, 10, pp.4266-4271, October, 2023

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

TOXICITY IMPACTS OF SILK DYE WASTE ON REPRODUCTIVE SYSTEM OF SWISS ALBINO MALE MICE MUS MUSCULUS AND ITS MITIGATION BY USING *MORINGA OLEIFERA* LEAF EXTRACT

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DOI: http://dx.doi.org/10.24327/ijrsr.20231410.0801

ARTICLE INFO

Article History:

Received 6th September, 2023
Received in revised form 21st September, 2023
Accepted 11th October, 2023
Published online 28th October, 2023

Keywords:

Silk dye waste, *Moringa oleifera* leaf powder, Histopathology of Testis, Testis Weight, Swiss albino male mice, toxicity assessment.

ABSTRACT

The environmental toxicology studies, the effect of environmental toxicant on the health of organisms and on the different compartments of the environments. Different scientific studies have been carried out over the years on the toxic effect of the silk dyes waste effluents on organisms. But current study has been premeditated to evaluate the effect of *Moringa oleifera* (Lam) leaf extract on albino male mice Mus musculas which is subjected to silk dye waste. The weight of testis and histopathology of testis have been taken on accounts. Five sets of animals i.e. Group I (Control), Group II (fed with 50% silk dye), Group III (fed with 100% silk dye), Group IV (mice fed with 50% dye treated with M. oleifera leaves powder), Group V (mice fed with 100% dye treated with M. oleifera leaves powder) have been taken for experiment. The dose of silk dye was 2ml/day to both groups II and III and M. oleifera leaf is given as per the standard dose (300mg/kg b.w) to both animals of group IV and V. The results show that the M. oleifera leaf extract when fed to Gr IV and V mice, demonstrated the regeneration of germinal epithelial cells and basement membrane, significant mitigation of spermatogenesis in different stage of testis when compared with mice of Gr- II and III. Then the M. oleifera leaf increased significant weight of testis of male mice when compared with Gr-II and III at 5% and 10% level of probability. This study suggested that the extract may have beneficial effect on histological sections of testis and weight of testis.

INTRODUCTION

Testis is the major organ for male sexual development and fertility. Sperm are produced in the testis. The testes have two interrelated functions as production of gametes (gametogenesis) and steroid (steroidogenesis). The seminiferous epithelium is composed of two basic cell types such as sertoli cells and spermatogenic cells. The spermatogenic cells further proliferated by the process of spermatogenesis: spermatogonial, spermatocyte and spermatids or spermatozoa.

The human are exposed to various type of environmental contaminants at different stage of their life span, widely held of them are harmful. Silk dye waste is one of the major sources of hazardous pollutants. Industrialization is a godsend of independent India but that is allied with hazardous effluents and discharges polluting the environment. Silk industry as textile provides an important economic stand to the artisans but the dye waste or spent wash arising from the manufacturing unit cause great menace ,if released in the open. Silk dye waste effluents are more toxic to environment than the domestic sewage. Bhagalpur (25˚17'N latitude and 86˚83'E longitude) is endowed with age old silk fabric and yarn production units. Here, the manufacturers use mostly synthetic dye such as azo dyes as colorant for their products. Azo dye forms the largest and most important Silk industry provides an important economic group of synthetic dyes (Mathur *et al*., 2005).

Moringa oleifera or drumstick tree is a tropical plant widely known to be of possible great medicinal values (Fahey, 2005; Paliwal *et al*., 2011). It is a plant native to India, Pakistan, Bangladesh and Afghanistan and grows up to 5 or 10 meters in height. *Moringa oleifera* is considered to be an important medicinal plant. It is being used as anti-ulcer, diuretic, anti-inflammatory and wound healing agent (Caceres *et al*., 1991; Udupa *et al*., 1994; Bassey *et al*., 2013).Its leaves are used as nutrional supplement and growth promoter because of significant presence of protein, selenium, calcium, phosphorus, β-carotene and γ-tocopherol in it (Nambiar and Seshadri, 2001; Lakshminarayana *et al*., 2005; Sanchez-Machado *et al*., 2006). The therapeutic use of Moringa leaves have been extensively studied in treatment of anti-toxicity and antioxidant (Khatun, 2017; Khatun and Varma, 2017). But no work has been done on its property to mitigate the damages induced by silk dye waste on

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histopathological observation on testis and sperm profile of a mammal. Hence the present work has been undertaken to study the impact of silk dye waste on different profiles of albino mice and their subsequent recovery by application of *Moringa oleifera* leaf powder. This study was therefore designed to investigation the effect of *Moringa oleifera* on silk dye waste induced Testis such as histology of testis and weight of testis in male mice *Mus musculus.*

MATERIALS AND METHODS

Animals: Experiment was performed on 6 to 8 weeks old healthy laboratory inbreed mice *Mus musculus* weighing about 25-30 grams. The animals were obtained from University Department of Zoology, Bhagalpur. Mice were reared and maintained at the animal house of University Dept. of Zoology, T.M.Bhagalpur University, and Bhagalpur under standard conditions and fed with nutritional diet and water

Collection of Plant material: *Moringa oleifera* leaf powder has been procured from own home product (with the help of ECHO Technical Note, By Beth Doerr and Lindsay Cameron, 2005, North Fort Myer, FL 33917, USA) Bhagalpur, Bihar, India.

Collection of silk dye waste: Silk dye waste effluents were collected directly from discharge point of silk dye industries of Nathnagar, Bhagalpur at regular interval.

Experimental Design: The mice were divided into 5 groups of 8 animals each. Gr-I (control mice), Gr-II (mice treated with 50% silk dye waste),Gr-III (mice treated with 100% silk dye waste), Gr-IV (mice fed with 50% dye treated with *M. oleifera* leaves powder), Gr-V (mice fed with 100% dye treated with *M. oleifera* leaves powder).

Dosage: The control group was given normal food and water. Silk dye waste was administered orally 2ml/day (Chaurasia *et al*, 2005) group II and III for 30 and 60 days duration. *M. oleifer*a leaf powder was also fed orally 300mg/kg b.w to both the group IV and V for 30 and 60 days exposure as per the method suggested by Chatterjee *et al*, 2013.

Biological assays: Histopathological observation of Testis and weight of testis.

Tissue processing and staining: After 30 and 60 days of experiment, mice were sacrificed and their organ were removed, were fixed in fixative and paraffinised, Haematoxylin-Eosin stained sections of testid were observed under light microscope (Pears, 1985) on 10X magnification.

STATISTICAL ANALYSIS

Data were analyzed using a one way ANOVA followed with a post hoc test (least square division test) using the SPSS for comparison between different treatments. Results were presented as mean ± S.E and differences were considered as significant when $p<0.05$ and $p<0.10$.

RESULTS

Histological study of Testis and weight of testis (gm/100g b.w) in different test group animals (Gr-I, Gr-II, Gr-III, Gr-IV and Gr-V) of 30 days and 60 days exposure periods have been shown in Photomicrograph (Plate: 1, 2, 3, 4 and 5) and histograph (Table-1. and Graph-1).

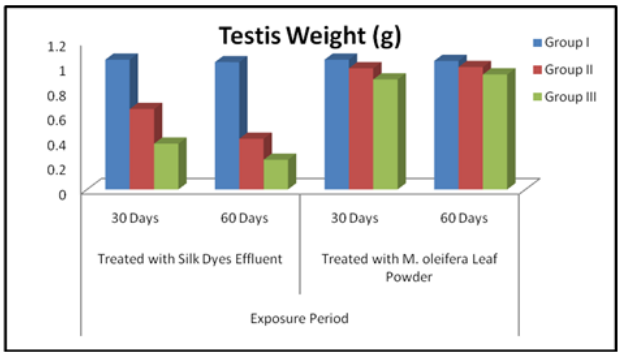
Testis Weight(g): The results show that the *M. oleifera* leaf extract when fed to Gr- IV and V mice, increase significantly the weight of testis at 5% and 10% level when compared with mice of Gr-II and III. This study suggested that the extract may have benificial effect on increasing weight of testis when treated with *M. oleifera* leaf powder.

Table: 1. Testis Weight (g/100g b.w) of mice after different exposure period among different Groups & their repair with *Moringa oleifera* leaf Powder

Experimental Groups	Exposure Period			
	Treated with Silk Dyes Effluent		Treated with *M. oleifera* Leaf Powder	
	30 Days	60 Days	30 Days	60 Days
Group I	1.05±0.289	1.03±1.98	1.04±0.87	1.05±0.98
Group II	0.65±3.89*	0.41±1.089*	0.98±2.98**	0.99±2.745**
Group III	0.37±3.06**	0.24±1.57**	0.89±0.09**	0.93±1.734*

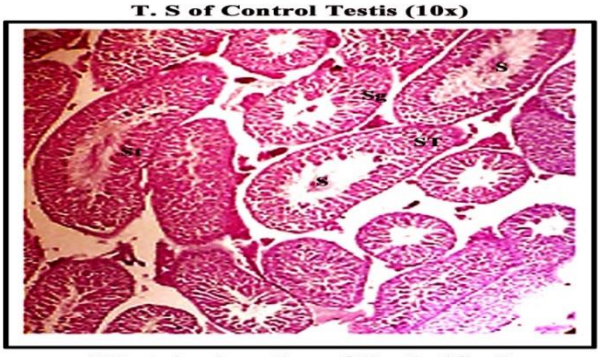
Note: based on the average of 10 individuals; ±Standard error of mean; value are statistically = *significant ($p<0.05$ and $p<0.10$) and **highly-significant, NS=Non-significant.

Graph 1 The graph of Testis weight (g)



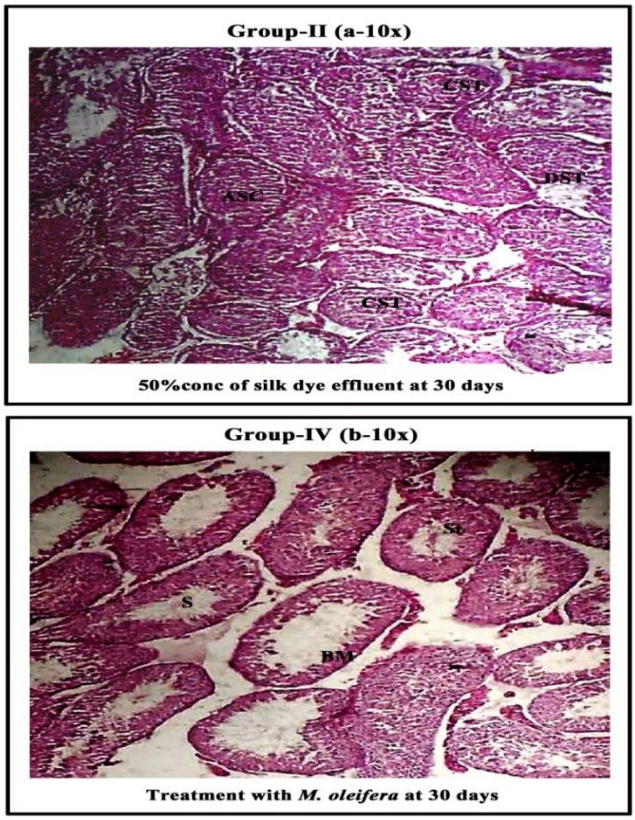
HISTOPATHOLOGICAL OBSERVATION OF TESTIS : Histopathological study on the testis of control (Gr-I) mice exhibit normal histoarchitecture with Sg, ST, St and S (Plate: 1). Group-II and III mice which were treated with 50% and 100% silk dye waste at 30 days showed numerous AST, CST, DST, DSC and DSt (Plate: 2.a and 3.c). Animals of Group-IV and V treated with *M. oleifera* leaves powder for 30 days showed regeneration of seminiferous lining cells, RS, RST, RSt, Sg, S, ST, St and BM (Plate: 2.b and 3.d). In case of Group-II and III treated with 50% and 100% silk dye waste at 60 days showed DBM, NVS, NVSt, DSg, ISg, ASt and AS (Plate: 4.e and 5.g). Group-IV and V treated with *Moringa oleifera* leaf extract at 60 days showed NS,ST, St, S, Sg, RST, RSg and RSt (Plate: 4.f and 5.h). The testis transverse Section of mice showed more or less normal tissue architecture..

Histopathological Photograph



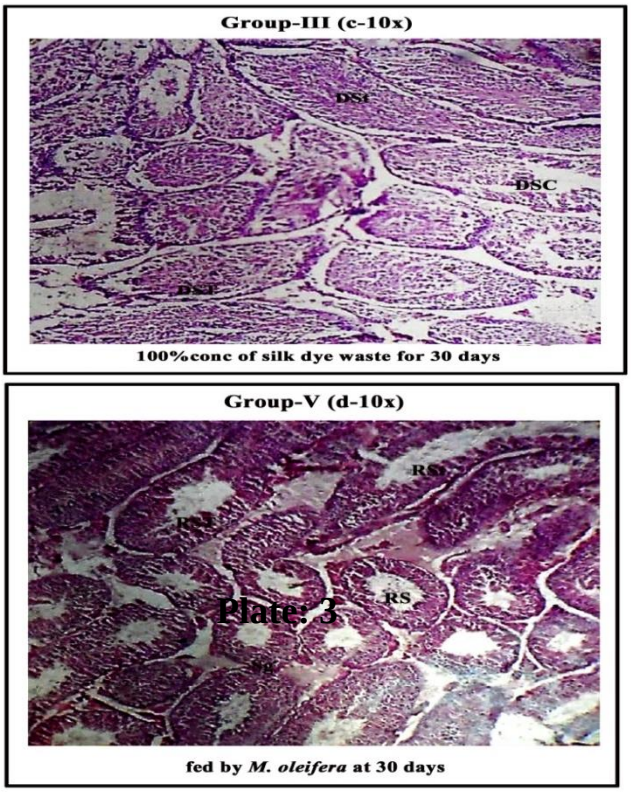
Caption: S- Spermatozoa, **ST**- Spermatocytes, **St**- Spermatids and **Sg**- Spermatogonial cells

Plate: 2



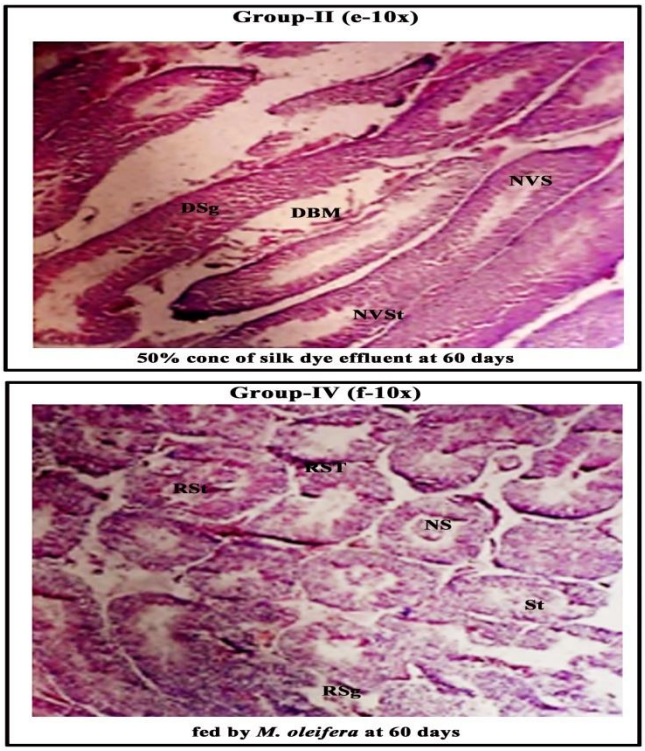
Caption: S- Spermatozoa, ST- Spermatocytes, St- Spermatids, BM- Basement Membrane, AST- Atrophied of Seminiferous cells and CST- Collapsed of Spermatocytes

Plate: 3



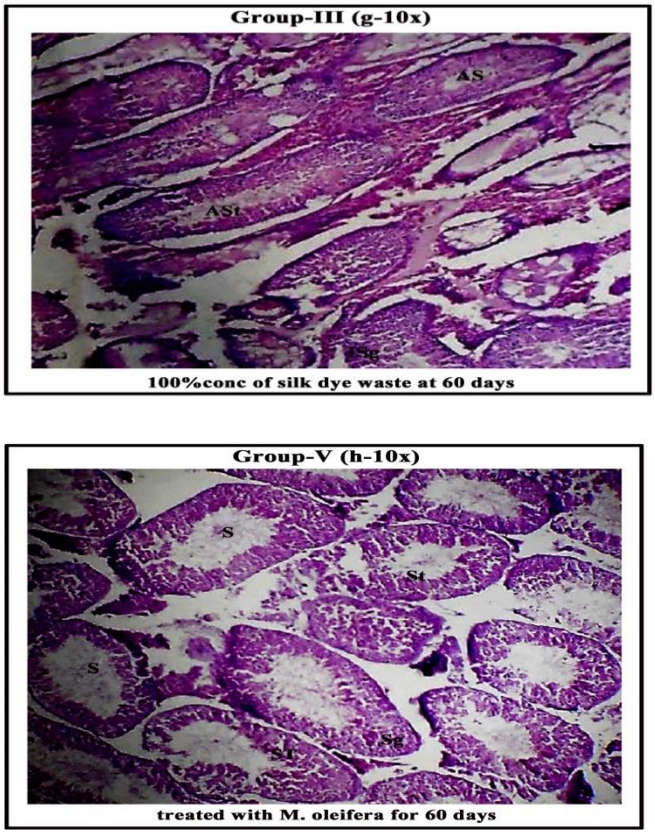
Captions: RS- Regeneration of Spermatozoa, **RST**- Regeneration of Spermatocytes, **RSt**- Regeneration of Spermatids, **DST**- Disrupted of Spermatocytes, **DSt**- Disrupted Spermatids **DSC**-Disrupted Sertoli cells and **Sg**- Spermatogonial cell

Plate: 4



Captions: RS- Regeneration of Spermatozoa, **RST**- Regeneration of Spermatocytes, **RSt**- Regeneration of Spermatids, **DST**- Disrupted of Spermatocytes, **DSt**- Disrupted Spermatids **DSC**-Disrupted Sertoli cells and **Sg**- Spermatogonial cell

Plate: 5



Captions: ST- Spermatocytes, **ASt**- Atrophy of Spermatids, **St**- Spermatids**, ISg**- Interrupted Spermatogonial cells, **Sg**- Spermatogonial cells, **S**- Spermatozoa and **AS**- Atrophy of Spermatozoa

DISCUSSION

The results show that the Moringa oleifera leaf extract when fed to Gr IV and V mice, showed the significant recovery of testis histology and weight of testis when compared with silk dye effluent induced mice of Gr- II and III after 30 and 60 days incubation period. In the present experimental study, the Testis of experimental mice exposed to silk dye waste (Gr-II and III) animals damage the testis weight and architecture when compared to control male mice (Gr-I). Susheela & Das (1988) reported the significant changes observed in the epithelial cells lining the ductuli efferent's of the caput epididymidis and vas deferens in the testis of fluoride treated animals. Bedford (1975); Orgebin-Crist *et al*, (1975); Prasad & Rajalakshmi (1976); Courot (1981); Eddy (1988) reported similar ultrastructure change including spermatogenic cells, vas deference damage in the testis of rats treated with benzoates, nickel and sulphured. Silk dye effluent induced change in ultrastructure of liver on swiss albino mice mus musculus and application of moringa oleifera leaf powder (Serina, K in 2022).

Alterations in the histology of both the caput and cauda epididymis of male mice treated with 10 and 20mg NaF/kg body weight for 30 days has been reported by Chinoy & Sequeira (1989). In human subjects suffering from endemic and industrial fluorosis a decrease in the sperm count and increase in the incidence of oligospermia and azospermia have been reported by Tarinsky, (1972) and Neelam et at, (1987). Susheela and Kumar (1991) reported that the change in the seminiferous epithelium and its spermatozoa of rabbits treated with 10 mg to 50mg NaF/kg body weight. Silk dye effluent induced change in ultra-structure of testis on Swiss albino male mice Mus musculus and recovery by Moringa oleifera leaves powder (Serina in 2023).

In the present study, the Moringa oleifera leaf extract has positive effect on testis weight and histoarchitecture in Gr- IV & V Swiss albino male mice when compared with animals of Gr- II and III. Flavonoid is a common constituent of Moringa and is well known antioxidants that can ameliorate oxidative stress-related testicular impairments in animal tissues (Ghose *et al*, 2004). Antioxidant also stimulates testicular androgenesis and is essential for testicular differentiation, integrity and steroidogenic functions (Hoskin *et al*, 1977; Kaur, 1980; Jowsey *et al*, 1972). Moringa oleifera leaf extract on silk dye waste induced histopathotoxicity on liver and testis of Swiss albino male mice Mus musculus reported by Khatun and Varma (2017). This histoarchitecture evidence in the present investigation was the clear indication of confirming the Spermatogenic efficacy of extracts of M. oleifera leaves in male albino rats. Histopathological study of the toxicity effect of silk dye waste on Kidney of Swiss albino mice Mus musculus and mitigation by using Moringa oleifera leaf powder (Khatun, 2017). Serina Khatun in 2017 also reported that the toxicity of silk dye waste on lung of Swiss albino male mice Mus musculus and its mitigation by using Moringa oleifera leaf extract.

Dym *et al*, (1979) reported that the numbers of mature leydig cells as well as number of spermatocytes and spermatids were significantly increased, which reflect the increase of androgen level. Similar findings were also reported in the study of Spermatogenic effect of Nigella sativa (Mukhallad *et al*, 2009) and Curculigo orchioides in male rats (Chauhan and Dixit, 2008). The Wistar rats those were treated with Moringa oleifera after alcohol administration however, showed a largely preserved testis weights, testis weight or body weight ratio and testis volumes (Ismail *et al*, 2007). Liver histopathological study of silk dye effluent induced Swiss albino mice Mus musculus and its mitigation by using Moringa oleifera leaf powder (Serina, K in 2023).

CONCLUSION

This study concluded that the M. oleifera leaf powder significantly reduces the alteration arisen in testis weight and associated histological structures in the toxicity impact of silk dyes waste exposed male mice. Clearly, the present experimental study prove the benificial effect of Moringa oleifera leaf extract in protecting animals against silk dye effluent induced histopathological damage and weight of testis.

Acknowledgement

The author thanks to the honourable and sincere guide ex- Prof. and head, Dr. Manish Chandra Varma, University Department of Zoology, T. M. B. University, Bhagalpur for his cooperation.

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How to cite this article: Serina Khatun., 2023. Toxicity Impacts of Silk Dye Waste on Reproductive System of Swiss Albino Male Mice Mus Musculus and Its Mitigation by Using Moringa Oleifera Leaf Extract. *Int J Recent Sci Res.* 14(10), pp. 4266-4271.

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