|| Print ISSN: 2589-7837 || Online ISSN: 2581-3935 ||
International Journal of Medical Science and Diagnosis Research (IJMSDR)
Available Online at www.ijmsdr.com
NLM (National Library of Medicine ID: 101738824)
Volume 7, Issue 05; September-October: 2023; Page No. 01-11

Original Research Article

Silk Dye Effluent Induced Change in Ultra-Structure of Testis on Swiss Albino Male Mice *Mus Musculus* and Recovery by *Moringa Oleifera* Leaves Powder

Dr. Serina Khatun
University Dept. of Zoology, T.M.Bhagalpur University, Bhagalpur-812007, India

Received: 25-06-2023 / Revised: 15-07-2023 / Accepted: 25-08-2023
Conflicts of Interest: Nil
Corresponding author: Dr. Serina Khatun
DOI: https://doi.org/10.32553/ijmsdr.v7i5.989

Abstract:
The silk dye effluent is one of the most potential harmful chemicals liberated in the environment in an unexpected manner. Silk dye waste is widely used as a potent dyeing of yarn and fabrics in many countries and has been shown to produce some adverse health effects. This work focuses primarily on the effects of *Moringa oleifera* leaf extract on testis of silk dye effluent induced surface ultrastructure in Swiss albino mice *Mus musculus*. The testis has been taken an account for surface ultrastructural study. The mice were divided into 5 Groups 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. Administration of silk dye waste result were demonstrated disrupted spermatids, cessation of seminiferous tubule cell, irregular cavity, lump of seminiferous tubules, disrupted interstitial cell and occupied spermatogonial cells but used of *Moringa oleifera* leafs powder it was significantly recovered the damage tissues has been observed. This study suggested that the extract may have beneficial effect on surface ultrastructural constituents such as Testis.
Key words: Silk dye Effluent, *Moringa oleifera* leaf powder, Testis, *Mus musculus*, SEM for Ultra-Structure, Toxicity assessment.

Introduction

Ultrastructure is the architecture of cells that is visible at higher magnifications than found on a standard optical electron microscope. Surface ultrastructure can also be viewed with SEM as a standard histology technique. Such cellular structure as organelles, which allow the cell to function property within its specified environment, can be examined at the ultrastructure, along with molecular phylogeny, is a reliable phylogenetic way of classifying organisms (Laura *et al*, 2006).

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.

Silk dye waste is one of the major sources of hazardous pollutants. Industrialization is a boon of independent India but that is allied with hazardous effluents and discharges polluting the environment. Silk industry 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). Meyer in 1981 reported that the chemical structure of azo benzene and azo napthol derivatives.

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 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 investigate the effect of *Moringa oleifera* on silk dye waste induced ultra-structure of testis in albino male mice.

Materials and Method

Experimental animals: Experiment was performed on 6 to 8 weeks old healthy laboratory inbred male *Mus musculus* weighing about 30 to 35 grams. The animals were obtained from University Department of Zoology, Bhagalpur. Mice were reared and maintained at the animal house of University Department 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. 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. oleifera* 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:

Surface ultra-structure study of Testis on silk dye waste induced mice *Mus musculus* and their mitigation by using medicinal or herbal plant as *Moringa oleifera* leaf powder.

Ultra-structure process of SEM:

The experimental mice were sacrificed and their organs were removed and rinsed in buffer. The tissues were then fixed in 2.5% Glutaraldehyde. The tissues were again rinsed in buffer with Tween 80 solution for extra mucus removed. The tissues were finally fixed in 2.5% Glutaraldehyde for 24 hours at 4˚C. The tissues were dehydrated in graded acetones. Then tissues were dried by critical point method. The tissues were cemented to metal stub and gold coated to a thickness of approximately 20 nm. The tissues were examined under Scanning Electron Microscope (SEM).

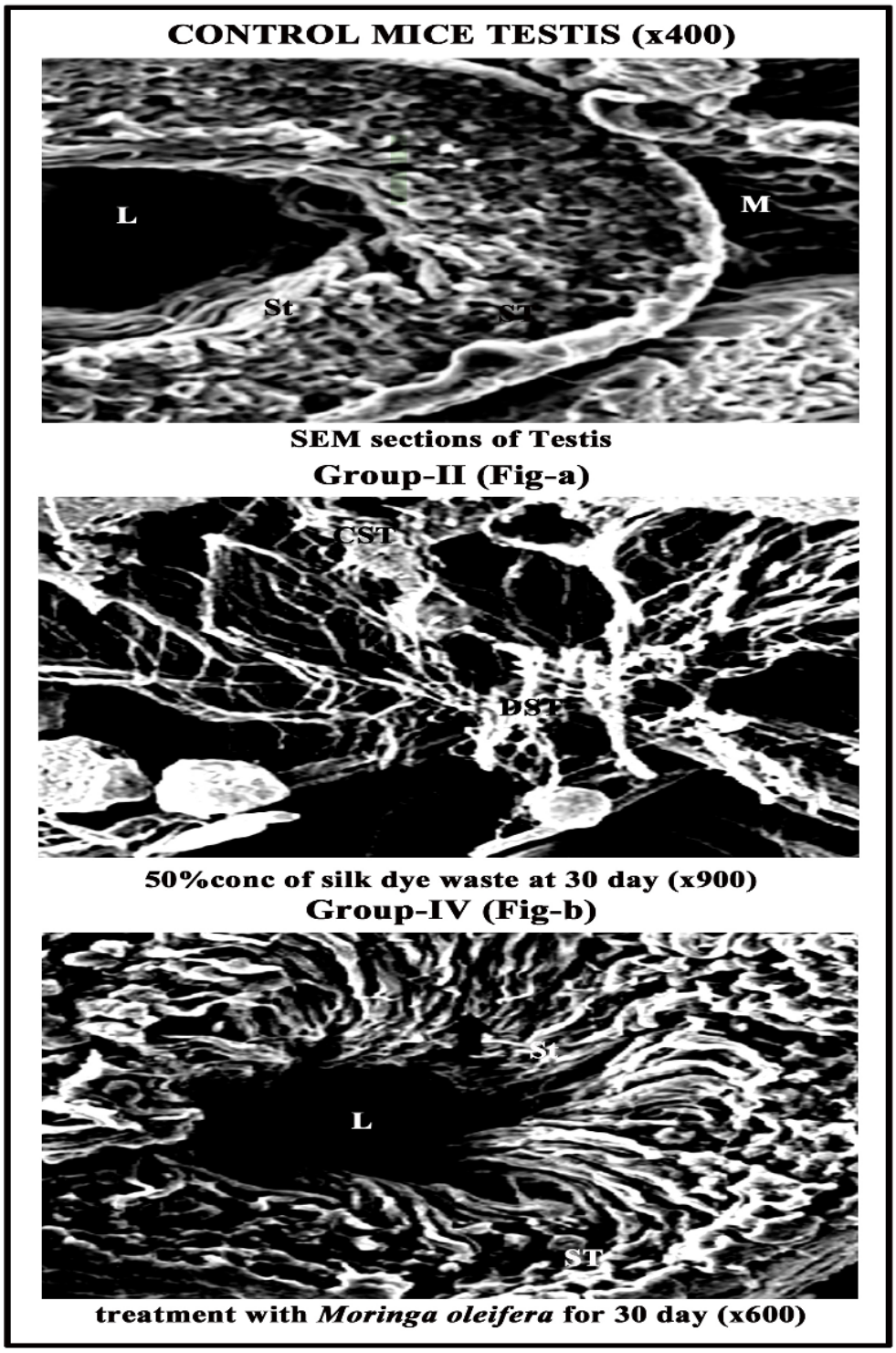
Result

Study of surface ultra-structure of Testis of different test group animals (Gr-I, Gr-II, Gr-III, Gr-IV and Gr-V) of 30 days and 60 days exposure periods by Scanning Electron Microscope (SEM), have been shown in Photomicrograph (Plate: 1, 2, 3 and 4).

Scanning electron microscopic study in control (Group-I) mice showed that the S are attached to the inner aspect of the L (Fig-1). In animals treated for 30 days with 50% silk dye effluent showed cessation of spermatogenesis and CST were devoid of S, DSt (Fig.-1.**a**). Group-IV mice showed significant repair of ST and St (Fig.-1.**b**).In case of Group-III treated showed the disrupted spermatogenic cells and a LST (Fig.-2.c).Group-V treated showed a significant improvement of testicular structure, St were found to have well defined lumen and tailed S (Fig.-2.**d**). In case of Gr-II treated with 50% silk dye waste for 60 days showed seminiferous tubules with flat M, IC, DST and IS (Fig.-3.**e**). Group-IV treated with *M. oleifera* leaf extract at 60 days showed RC and M, regeneration of ST and IS, repaired spermatogenic cell layers and improvement in the irregular outline of the St (Fig.-3.**f**).Upon treatment with 100% silk dye effluent (Group-III) after 60 days showed compressed seminiferous tubules with WBM. Some of the tubules are appearing empty while other OSC (Fig.-4.**g**). Animals of Group-V treated with *M. oleifera* leaf extract for 60 days showed like the more or less control mice as seminiferous tubules clearly indicated different stages of spermatogenesis, BS were seen in the lumen (Fig.-4.**h**).

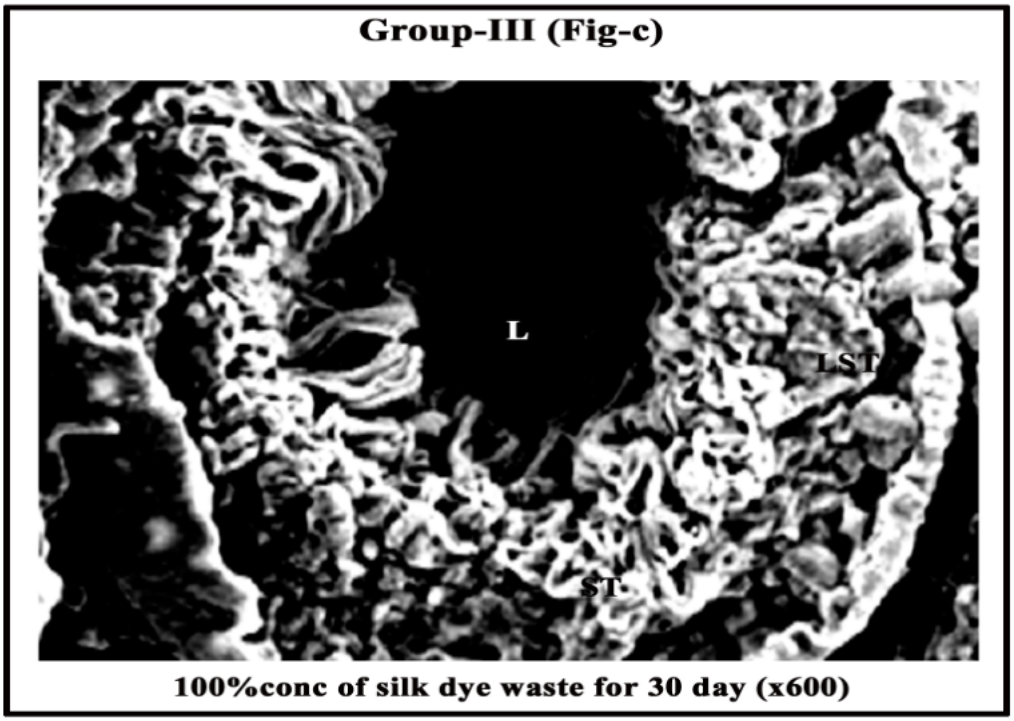
Photography of Testis in SEM:

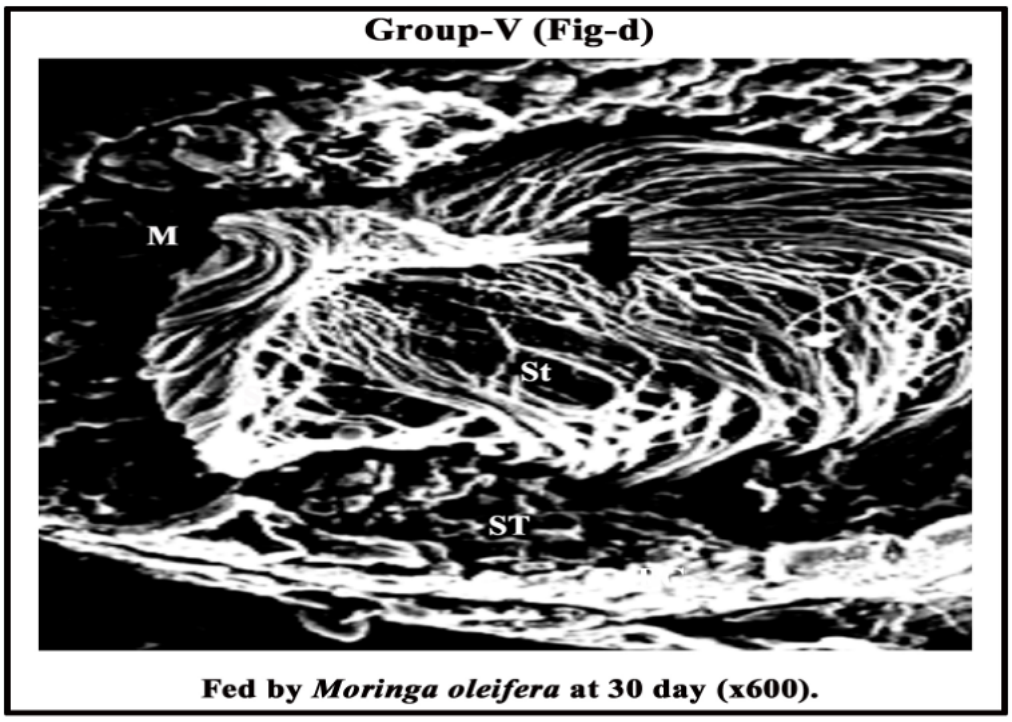
Plate: 1



Captions: ST- Spermatocytes, **St**- Spermatids, **M-** Myoid cell, **L**- Lumen of seminiferous tubules, **DSt-** Disrupted Spermatids and **CST**- Cessation of Seminiferous Tubule cells

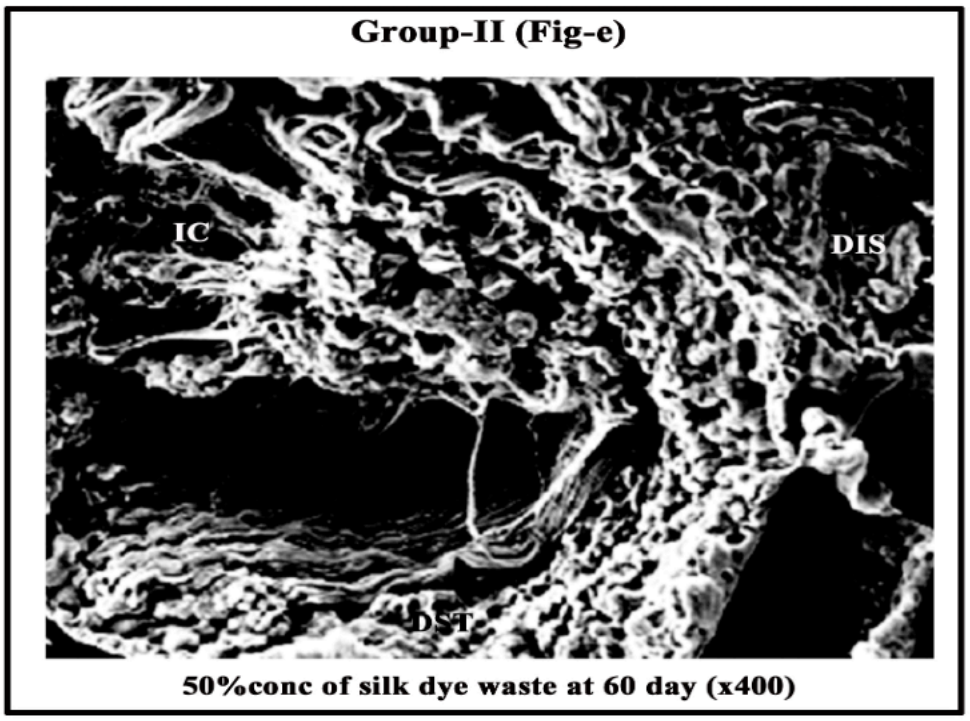
Plate: 2

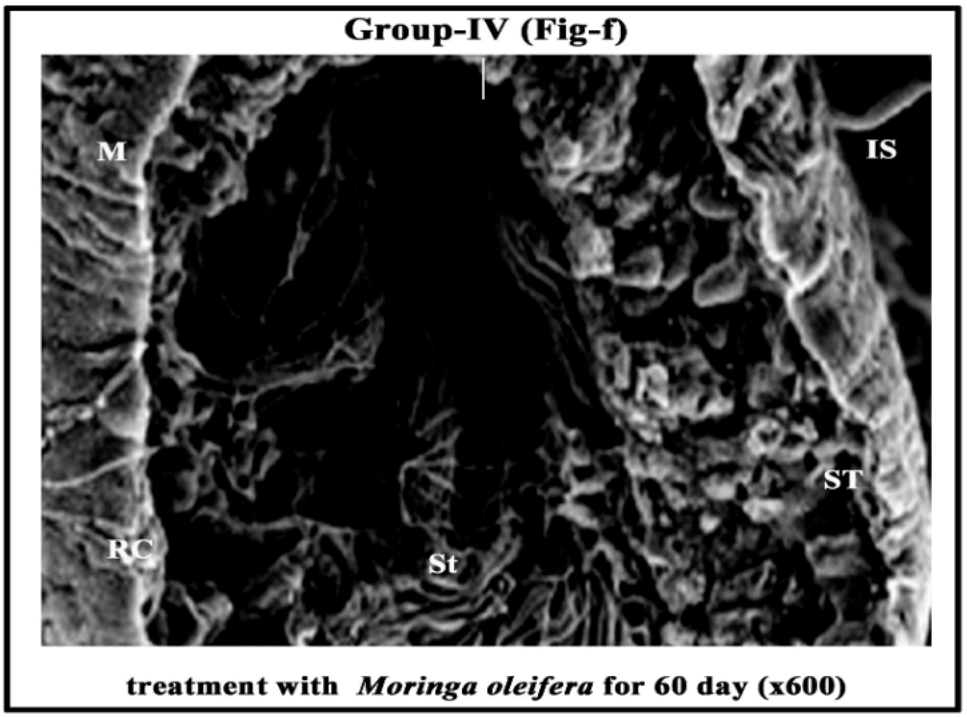




Captions: L- Lumen of seminiferous tubules, **ST-** Spermatocytes, **St-** Spermatids, **M**- Myoid cell, **LST-** Lump in Seminiferous Tubules and **C-** Connective tissue

Plate: 3

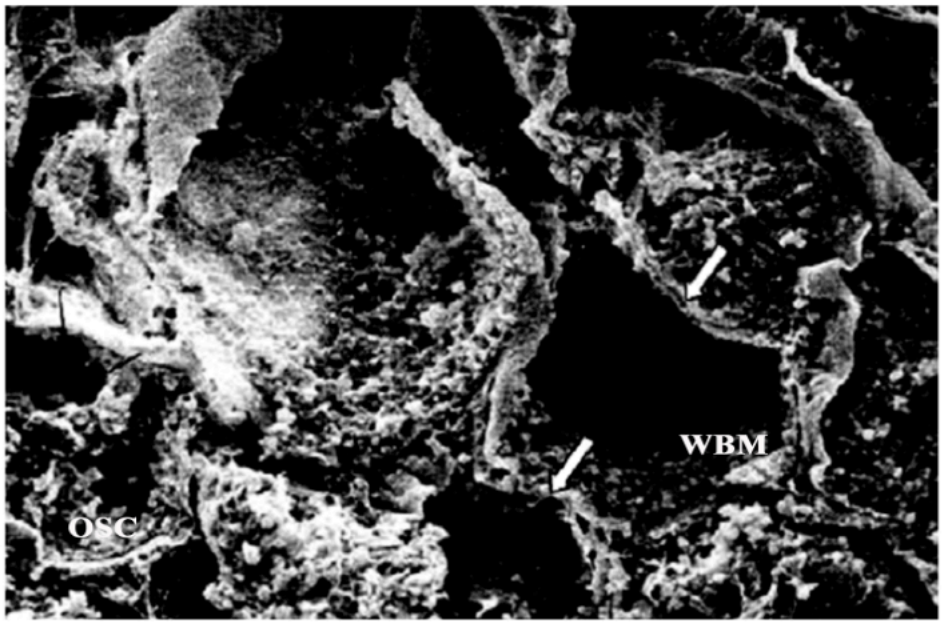




Captions: DST- Disrupted Spermatocytes, **DIS**- Disrupted Interstitial cell, **IC-** Irregular Cavities, **M**- Myoid cell, **ST**- Spermatocytes, **St**- Spermatids, **RC**- Regular Cavities and **IS-** Interstitial cell

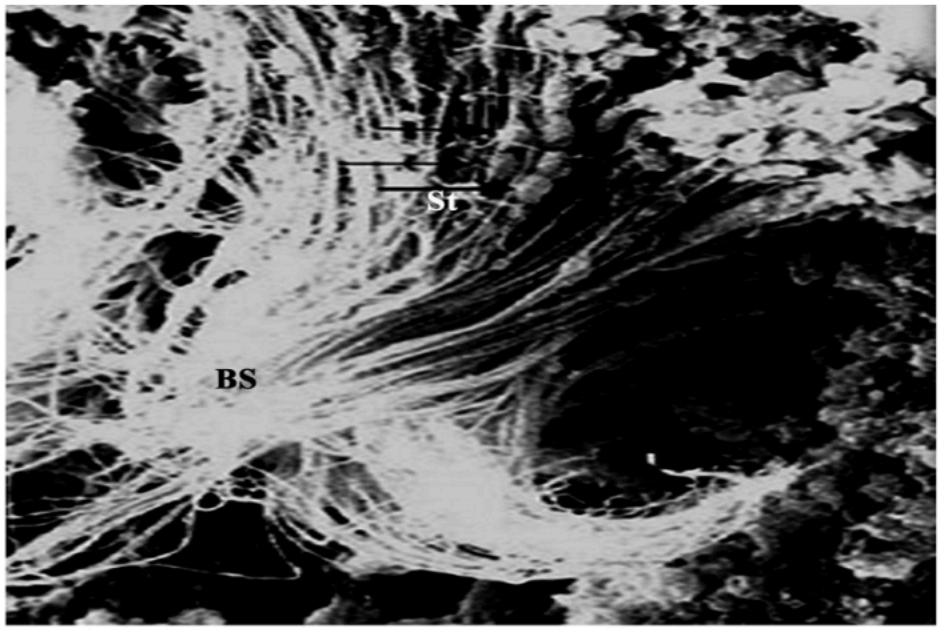
Plate: 4

Group-III (Fig-g)



100%conc of silk dye waste for 60 day (x400)

Group-V (Fig-h)



Fed by *Moringa oleifera* at 60 day (x900)

Captions: OSC- Occupied Spermatogonial cells, **WBM-** Wrinkle Basement Membrane, **St-** Spermatids and **BS-** Bundle of Spermatozoa

Discussion :

The results showed that the *M. oleifera* leaf extract when fed to Gr IV and V mice, resulted into the significant recovery of testis ultra-structure when compared with silk dye effluent exposed mice (Gr- II and III).

Testis of experimental mice exposed to silk dye waste (Gr-II and III) animals damage the testis 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.

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.

In the present study, the *Moringa oleifera* leaf extract has positive effect on testis 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).

Conclusion:

This study concludes that the *M. oleifera* leaf powder significantly reduces the damages arisen in the ultra-structure of testis of experimental mice which were due to the toxicity impact of silk dyes.

References

1. Bassey, R.B; Bala, D.N; Edagha, I.A and Peter, A.I (2013): The effect of ethanolic extract of Moringa oleifera on alcohol-induced testicular histopathologies in pre-

pubartal albino Wistar rats. ***Biology and Medicine***, 5: 40-45.

2. Bishayi B, Sengupta M, Ghosh S. Lead induced modulation of splenic macrophage responses on humoral and cell mediated immunity. Acta Microbiologicaet Immunologica Hungarica. 2004;51:31-45.
3. Bedford J.M. (1975) Maturation, transport and fate of spermatozoa in the epididymis. In Handbook of Physiology. Volume 5, Male reproductive system. Section 7, Endocrinology. Eds D.W. Hamilton & R.O. Greep. Washington,DC: American Physiological Society. pp. 303-317.
4. Caceres, A; Cabrera, O; Morales, O; Mollinedo, P. and Mendia, P (1991): Pharmacological properties of M. oleifera. 1: preminary screening for antimicrobial activity. ***Journal of Ethnopharmacology***, 33(3):213-216.
5. Chatterjee, P.K; Vinodini, N.A; Anwar, A; Nayanatara, A.K' Pal, S.R and Suman, V.B (2013): The front end in anti-electrostatic discharge for production, innovation, development: Polycarbonate/grapheme Composite. ***Int. J. of Inv in Sci. Eng. And Tech***, 2(9): pp- 4724-4734.
6. Chaurasia, O.P; Kumar, A. and Kumari, M. (2005): Genotoxic effect of silk dyeing waste in bone marrow cells of mice. Mus musculus, ***Cytologia,*** 70(4): 381-384.
7. Chinoy N.J. & Sequeira E. (1989a) Effects of fluoride on the histoarchitecture of reproductive organs of male mouse. Reprod. Toxicol. 3, 261-267.
8. Chinoy N.J. & Sequeira E. (1989b) Fluoride induced biochemical changes in reproductive organs of male mice. Fluoride 22,78-85.
9. Courot, M. (1981) Transport and maturation of Spermatozoa in epididymis of mammals. Prof. Reprod. Biol. 8, 67-69.
10. Das T.K., Susheela A.K., Gupta, I.P., Dasarathy S. & Tandon R.K. (1994) Toxic effects of chronic fluoride ingestion on the upper gastrointestinal tract. J. Clin Gastroenterol. 18, 194-199.
11. Eddy E.M. (1988) The spermatozoon. In The Physiology of Reproduction. Volume I. Eds E. Knobil & J.D. Neil. New York: Raven Press. pp. 27-68.
12. Elhalwagy, M.E.A; Darwish, N.S. and Zaher, E.M. (2008): Prophylactic effect of green tea polyphenols against liver and kidney injury induced by fenitrothion insecticide. ***Pestic. Biochem. Phys,*** 91:81-89.
13. Fahey, J. W; Beth Doerr and Lindsay Cameron (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. ***Trees for Life Journal***.
14. Gardner, G.H; Grove, R.C; Gustafson, R.K; Maire, E.D; Thompson, M.J; Wells, H.S. and Lamson, P.D. (1925): Studies on the pathological histology of experimental carbon tetrachloride poisoning. ***Bull. Johns. Hopkins. Hosp***. 36:107-133.
15. Gagliano, N.; Grizzi, F and Annoni, G (2007): Mechanism of aging and liver functions. ***Digest. Dis. Sci***., 25: 118-123.
16. Grewal, K.K; Sandhu, G.S; Ranjit Kaur; Brar, R.S and Sandhu, H.S (2010): Toxic impact of Cypermethrin on Behavior and Histology of certain tissues of albino rats. ***Toxicol Int,*** 20: 61-67.
17. Gokcimen, A; Gulle, K; Demirin, H; Bayram, D; Kocak, A and Altuntas, I (2007): Effect of diazinon at different doses on rat liver and pancreas tissues. ***Pesticide Biochem. Physiol,*** 87: 103-108.
18. Heileh, S; Therasa, S.C (2008): M. oleifera polyphenols down regulates cyclooxygenase and Bcl-2 activity in acetaminophen induced hepatotoxicity. ***Springer Sciences Media, LLC.***
19. Hoskins D.D., Johson D., Brandt H. & Ascott T.S. (1979) Evidence for a role for a forward motility protein in the epididymal development of sperm motility. In The Spermatozoon: Maturation,

Motility, Surface Properties and Comparative Aspects. Eds D.W. Fawcett & J.M. Bedford. Baltimore: Urban & Schwarzenberg. pp. 43.

20. Jowsey J., Riggs B.L., Kelly P.J. & Hoffman P.L. (1972) Effects of combined therapy with sodium fluoride, vitamin D and calcium in osteoporosis. Am. J. Med. 53, 43-49.

21. Kaur K. & Singh J. (1980) Histological finding of mice testes following fluoride ingestion. Fluoride 13, 160-162.

22. Ismail, M; Wang, O; Huang, X; Cao, L; Chen, X; Sun, K; Liu, Y; Li, W. and Zhang, L (2007): Green tea polyphenol epigallocatechin-3-gallate inhibits oxidative damage and preventive effects on carbon tetrachloride induced hepatic fibrosis. ***J. Nutr. Bioch.*** 18:795-805.
23. Khatun S. (2017, April). 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., ***Advance Research Journal of Multidisciplinary Discoveries***.12.0,C-1(2017):01-04
24. Khatun. S and Varma. M.C (2017): Role of *Moringa oleifera* leaf extract on silk dye waste effluent induced histopathotoxicity on liver and testis of Swiss albino male mice Mus musculus. ***IOSR Journal of Pharmacy***, 7: 4(1); Pp- 1-4.
25. Lakshminarayana, R; Raju, M; Krishnakantha, T.P and Baskaran, V. (2005): Determination of major carotenoids in a few Indian leafy vegetables by high-performance liquid chromatography. ***Journal of Agricultural and Food Chemistry***, 53(8):2838-2842.
26. Mathur, N; Mehta, M; Chaudhry, V (2005): Sperm abnormality Induction by Food colour metanil yellow. ***J. Ecolphysiol. Occup. Hlth***; 5:157-160.
27. Meyer, U. (1981): Bio degradation of synthetic organic colorants. FEMS Symp; 12:371-385.
28. Mukhallad , V.; Muthuraman, P.; Hawang, I. and Srikumar, K. (2009): Biochemical and Histopathological changes induced by low doses of carbendazin on testis of male albino rat. International Journal of Pharmacy and Biological Sciences, 1(4): 572-576.
29. Muthukumaran, S, Sdheer, A.R; Menon, V.P. and Nalini, N (2008): Protective effect of quercetin on silk dye induced pro-oxidant and antioxidant imbalance and DNA. 13(5):217-224.
30. Neogy, S; Mahapatra, S; Mandal, S.K. and Somenath, R (2008): Amelioratory effect of Andrographis paniculata Neem on liver, kidney, heart, lung and spleen during nicotine induced oxidative strees. ***Environ. Toxicol. Pharma***. 25:321-328.
31. Neelam, K., Suhasini, R.V. & Sudhakar, R.Y. (1987) Incidence on prevalence of infertility among married male members of endemic fluorosis district of Andhra Pradesh. In Proceedings of a Conference of the International Society for Fluoride Research. Switzerland (Nyon) (Abstract).
32. Nambiar, V.S and Seshadri, S(2001): Bioavailability trials of β-carotene from fresh and dehydrated drumstick leaves(*M. oleifera*) in a rat model. ***Plant Food for Human Nutrition,*** 56(1):83-95.
33. Orgebin-Crist M.C., Danzo B.J. & Davies J. (1975) Endocrine control of the development at maintenance of sperm fertilizing ability in the epididymis. In Handbook of Physiology, Volume 5, Male reproductive system. Section 7, Endocrinology Eds. W.D. Hamilton & R.O. Greep. Washington, DC: American Physiological Society. pp. 319.
34. Ogura, R; Ikeda, N; Yuki, K; Morita, O; Saigo, K; Blackstock, C; Nishiyama, N. and Kasamatsu, T (2008): Genotoxicity studies on *M. Oleifera* leaves powder. ***Food Chem. Toxicol.*** 46:2190-2200.
35. Paliwal, R.; Sharma, V.; Pracheta; Sharma, S.; Yadav, S. & Sharma, S. (2011). Anti-

nephrotoxic effect of administration of *Moringa oleifera* Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss Albino mice. ***Biol. Med****., 3(2)*:27-35.
36. Prasad M.R.N. & Rajalakshmi M. (1976) Comparative physiology of the epididymis. Gen. Comp. Endocrinol. 28, 530-537.
37. Roganovic, Z.D. and Jordanova, M. (1998): Liver lesions in bleak collected from some contaminated sites on lake Ohrid. A Histopathological evidence. ***Ekol. Zast. Zivot. Sred.***6:11-18.
38. Sanchez-Machado DL, Lopez-Cervantes J, Vazquez NJ, 2006. High-performance liquid chromatography method to measure α and γ-tocopherol in leaves flowers and fresh beans from M. oleifera. ***Journal of Chromatography A***, 1105(1-2):111-114.
39. Dym, P. B: (1979): Dimethoate- induced biochemical and histopathology change in the liver of rats. ***Exp. Toxicol. Pathol***, 59: 237-243.
40. Serina, K. (2017): Toxicity of silk dye waste on lung of Swiss albino male mice *Mus musculus* and its mitigation by using *Moringa oleifera* leaf extract. ***Inter. J. of Current. Adv. Res,*** 6(3), March, 2657-2660.
41. Selmanoglu, G. and Akay, M.T. (2000): Histopathological effects of the pesticide combinations on liver, kidney and testis of male albino rats. ***Pesticides,*** 15:253-262.
42. Susheela A.K. & Kumar A. (1991) A study of the effect of high concentrations of fluoride on the reproductive organs of male rabbits, using light and scanning electron microscopy. J. Reprod. Fertil. 92, 353-360.
43. Tarinsky, F; Zhou, Y; Laura, S. and Chen, A. (1972): The antifibrogenic effect of epigallocatechin gallate results from the induction of de novo synthesis of glutathione in passaged rat hepatic stellate cells. ***Lab. Invest***. 86:697-709.
44. Varilek, G.W; Yang, F; Lee, E.Y; DeVilliers, W.J; Zhong, J; Oz, H.S; Westberry, K.F. and McClain, C.J (2001): *Moringa oleifera* polyphenol extract attenuates inflammation in interleukin-2-deficient mice, a model of autoimmunity. ***J. Nutr****.* 131(7):20-34.
45. Yildiz, D. (2004): Nicotine, its metabolism and an overview of its biological effects. ***Toxicon.*** 43:619-632.
46. Yousef, M.I; Demerdash, F.M; Kamel, K.I. and Al-Salhen, K.S. (2003): Changes in some haematological and biochemical indices of rabbits induced by isoflavones and cypermethrin, ***Toxicology,*** 189:223-224.
47. Udupa, S.L; Udupa. A.L and Kulkrni, D.R. (1994): Study on the anti-inflammatory and healing properties of *M. oleifera* and marmelos. ***Fitoterapia***, 65(2):119-123.