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TOXICITY IMPACTS OF SILK DYE WASTE ON SPERM PARAMETERS AND HISTOPATHOLOGY OF TESTIS OF SWISS ALBINO MALE MICE *MUS MUSCULUS* AND ITS MITIGATION BY USING *MORINGA OLEIFERA* LEAF EXTRACT

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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 sperm profile 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 significantly number of sperm count and % of sperm motility but decrease the % of sperm mortality of 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 sperm count, mortality and motility.*

KEYWORDS: Silk Dye Waste, Moringa Oleifera Leaf Powder, Histopathology, Testis, Sperm Parameters, Swiss Albino Male Mice, Toxicity Assessment.

INTRODUCTION

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 antiulcer, diuretic, anti-

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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 hyperglycemia (Priyadarshani, 2015; Pankaj et al, 2013), anti-toxicity (Khatun et al, 2016 & 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 histopathology of testis and sperm profile in albino male mice.

MATERIALS AND METHOD

Animals: Experiment was performed on 6 to 8 weeks old healthy laboratory inbred male *Mus musculus* weighing about 30 -35 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 10 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. 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: Histopathological observation of testis and sperm parameters as sperm count, sperm mortality and sperm motility.

Sperm counting: The cauda epididymides were incised and the sperm were allowed to swim for 15 minutes. Solution of 1:10 dilution is made by adding 90 ml of distiled water to 10ml of sperm suspension. Sperm counts were done by using haemocytometer (Wyrobek, 1997).

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 testis were observed under light microscope (Pears, 1985) on 40X magnification.

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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 $p<0.10$.

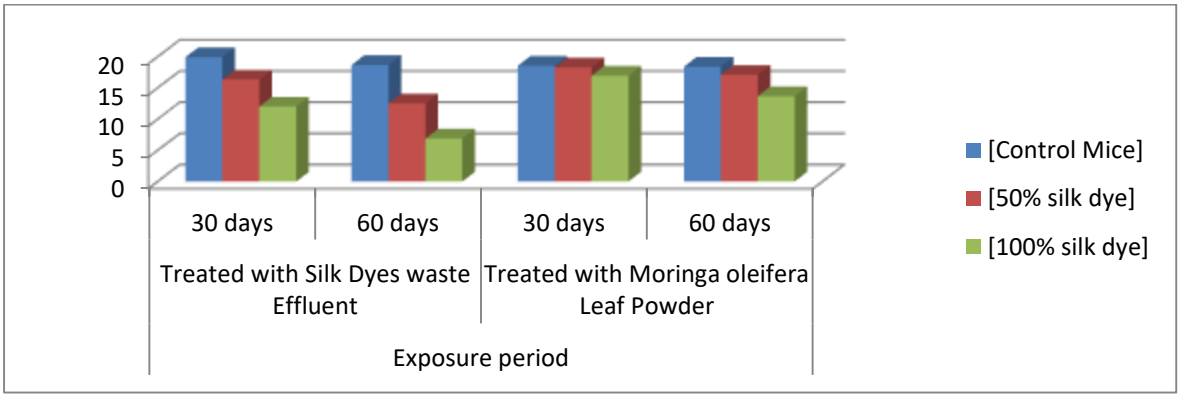
RESULTS

The results show that the *M. oleifera* leaf extract when fed to Gr- IV and V mice, increase significantly the sperm count, sperm motility at 5% and 10% level where as marked decrease in the sperm mortality ($p<0.05,0.10$) when compared with mice of Gr-II and III. This study suggested that the extract may have benificial effect on histological section of testis when treated with *M. oleifera* leaf powder.

Study of Sperm Profile:

Table.1. Reproductive parameter [Sperm count (million per cubic mm)] of mice after different exposure period among different Groups & their repair with *Moringa oleifera* leaf Powder.

Experimental Groups	Exposure Period			
	Treated with Silk Dyes waste Effluent		Treated with M. oleifera Leaf Powder	
	30 Days	60 Days	30 Days	60 Days
Group I	19.92±0.00	18.68±0.00	18.54±0.00	18.40±0.00
Group II	16.32±0.04	12.51±0.00	18.28±0.00	17.09±0.00
Group III	12.00±0.00	6.88±0.00	16.94±0.00	13.67±0.00



Graph:-1. Showed the total sperm count (million/mm^3).

Table.2. Reproductive parameter [Sperm Motility (%)] of mice after different exposure period among different Groups & their repair with *Moringa oleifera* leaf Powder.

Experimental Groups	Exposure Period			
	Treated with Silk Dyes waste Effluent		Treated with M. oleifera Leaf Powder	
	30 Days	60 Days	30 Days	60 Days
Group I	98.66±0.01	90.52±0.15	93.02±0.01	92.66±0.00
Group II	81.14±0.00	60.26±0.00	90.99±0.00	85.60±0.01
Group III	58.52±3.78	33.93±0.79	81.34±0.00	65.89±0.09

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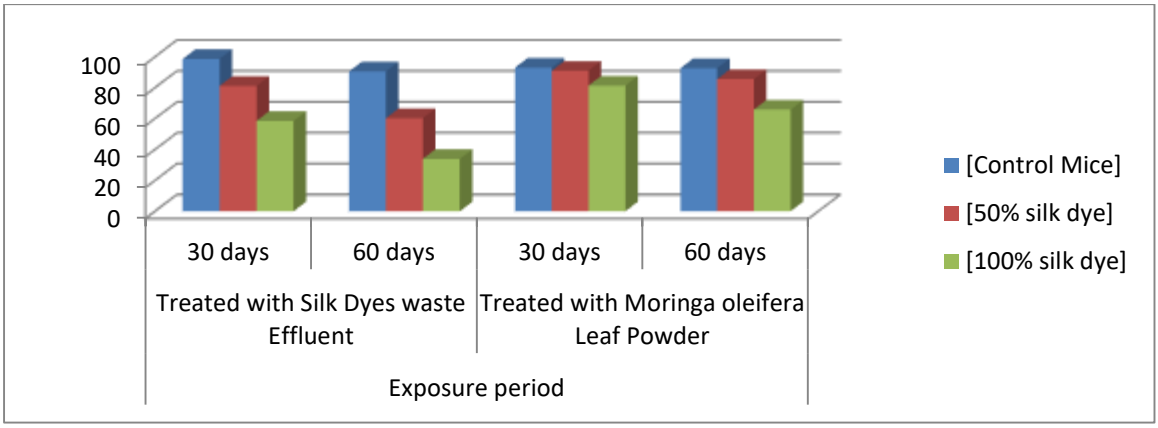
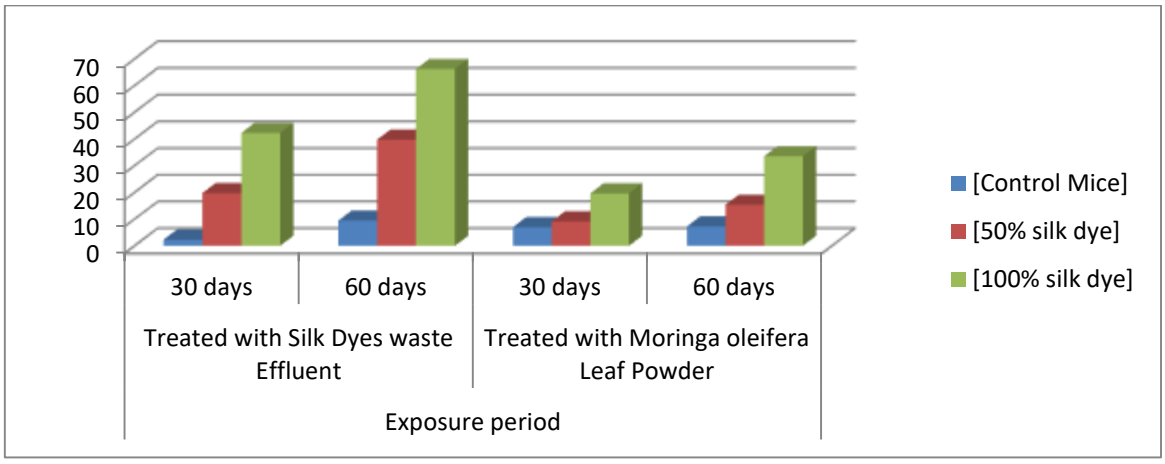


Graph:-2.Showed the total sperm motility (million/mm³).

Table.3. Reproductive parameter [Sperm Mortality (%)] of mice after different exposure period among different Groups & their repair with *Moringa oleifera* leaf Powder.

Experimental Groups	Exposure Period			
	Treated with Silk Dyes waste Effluent		Treated with M. oleifera Leaf Powder	
	30 Days	60 Days	30 Days	60 Days
Group I	2.30±0.03	9.47±0.15	6.98±0.11	7.26±0.00
Group II	19.73±0.06	39.73±0.00	9.15±0.00	15.39±0.01
Group III	42.22±3.56	66.21±1.23	19.64±0.00	33.5±0.27



Graph:-3.Showed the total sperm mortality (million/mm³).

Histopathological observation of Testis: Histopathological study on the testis of control (Group-I) mice showed normal histoarchitecture with blood capillaries, spermatogonial cells, spermatocyte cells, spermatids and spermatozoa (Fig-1). In case of Group-II treated with 50% silk dye waste at 30 days showed numerous atrophied and collapse of seminiferous living cells and spermatocytes cells (Fig-2). Group-IV treated with *M. oleifera* leaves powder at 30 days showed regeneration of seminiferous living cells and spermatocytes cells (Fig-3).Treated with 100% silk dye waste (Group-III) at 30 days showed the spermatogenic cells were disrupted, atrophy of sertoli cells and degeneration of seminiferous living cells (Fig-4). Group-V treated with *M. oleifera* leaf extract at 30 days showed regeneration of seminiferous living cells,

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amelioration of spermatogenic cells and more or less sertoli cells (Fig-5). In case of Group-II treated with 50% silk dye waste at 60 days showed disrupted of seminiferous tubules, spermatogonial cells, spermatocytes cells, mess up of connective tissues and no clear vision of spermatozoa and spermatids (Fig-6). Group-IV treated with *Moringa oleifera* leaf extract at 60 days showed narrowed spermatozoa and spermatids, regeneration of connective tissues, spermatogonial cells and spermatocytes (Fig-7). Treated with 100% silk dye waste (Group-III) at 60 days showed completely disrupted seminiferous living cells, interrupted spermatogonial cell, atrophy of spermatozoa and spermatids, worsening of blood vessel (Fig-8). In case of Group-V treated with *M. oleifera* leaf extract at 60 days showed regeneration of blood vessels, present of spermatozoa and spermatids. The testis section of mice showed more or less normal tissue architecture (Fig-9).

Group-I (Fig-1)

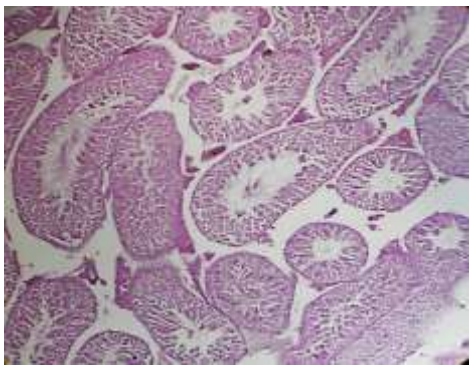
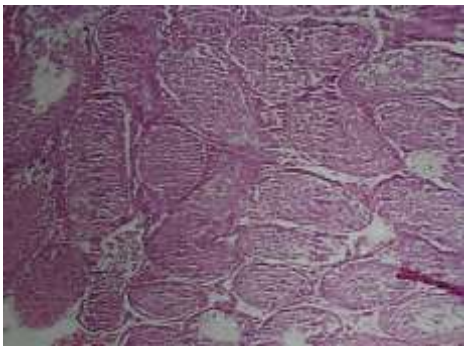
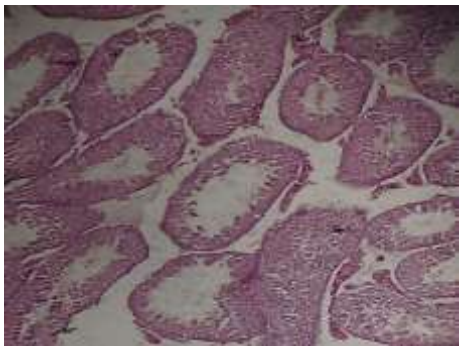


Figure:-1. Photomicrograph of testis section of male mice showed normal histoarchitecture. **(x40, H&E).**

Group-II (Fig-2)



Group-IV (Fig-3)



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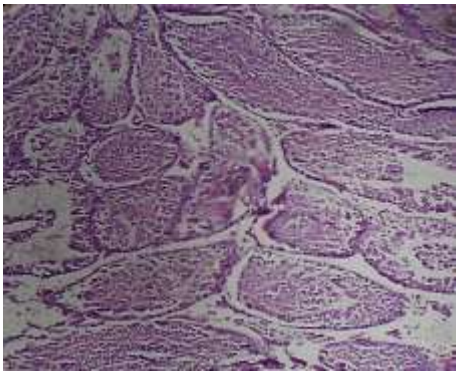
Group-III (Fig-4)	Group-V (Fig-5)
	

Figure:-2. Photomicrograph of testis section of mice showed, atrophied and collapses the seminiferous living cells. **(x40, H&E). Figure:-3.** Photomicrograph of testis section of mice showed, regeneration of seminiferous living cells and spermatocyte cells. **(x40, H&E). Figure:-4.** Photomicrograph of testis section of mice showed, disrupted of spermatogenic cell and atrophy of sertoli cell. **(x40, H&E). Figure:-5.** Photomicrograph of testis section of mice showed, regeneration of spermatogenic cells and amelioration of sertoli cells. **(x40, H&E).**

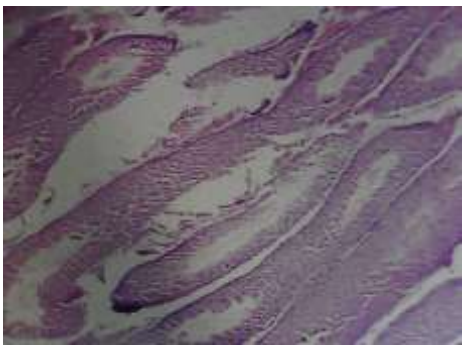
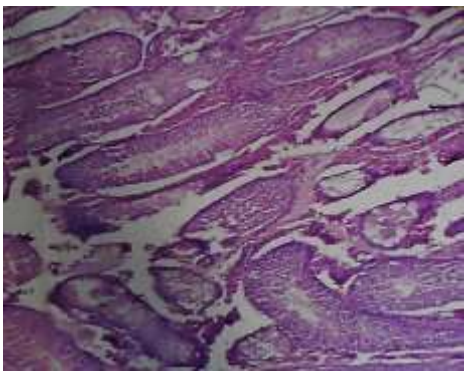
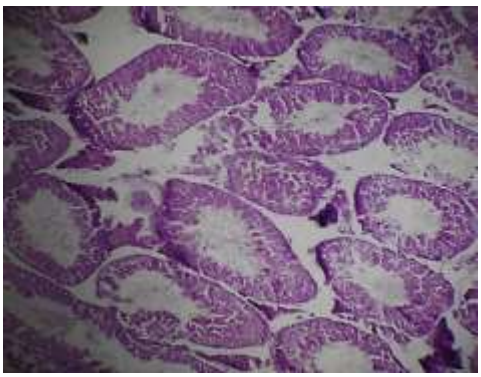
Group-II (Fig-6)	Group-IV (Fig-7)
	
Group-III (Fig-8)	Group-V (Fig-9)
	

Figure:-6. Photomicrograph of testis section of mice showed; disrupted of spermatogonial cells, spermatocytes cells, mess up of connective tissues and no clear vision of spermatozoa

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and spermatids. **(x40, H&E). Figure:-7.** Photomicrograph of testis section of mice showed, narrowed spermatozoa and spermatids, regeneration of connective tissues, spermatogonial cells and spermatocytes. **(x40, H&E). Figure:-8.** Photomicrograph of testis section of mice showed, interrupted spermatogonial cell, atrophy of spermatozoa and spermatids, worsening of blood vessel. **(x40, H&E). Figure:-9.** Photomicrograph of testis section of mice showed, regeneration of blood vessels, present of spermatozoa and spermatids. **(x40, H&E).**

DISCUSSION

The results show that the *M. oleifera* leaf extract when fed to Group IV and V mice, showed the significant recovery of Testis and Sperm parameters when compared with mice of Gr- II and III.

In this experimental study, testicular atrophy and distortions in spermatogenic cells were observed in groups treated with silk dye waste effluent. Many researchers have expressed their concern about the rising cases of male spermatozoa abnormalities (Kaku and Osegbe, 1989). Numerous studies have indicated that alcohol abuse in men can cause impaired testosterone production and testicular atrophy (Adler, 1992). Those changes can result in impotence, infertility and reduced male secondary sexual characteristics. Testicular atrophy results primarily from the loss of sperm cells and decreased dimemeter of the seminiferous tubules (Van Thiel et al, 1974). The vitamin-C also counteracts the testicular oxidative stress induced by exposure to pro-oxidant such as arsenic, PCBs, cadmium, endosulfan and alcohol (Sen Gupta et al, 2004; Senthil et al, 2004; Maneesh et al, 2005; Rao et al, 2005; Chang et al, 2007). Toxicity impact of silk dye waste induced reproductive system of male mice studied by Khatun et al, 2017. Mishra and Singh (2009) reported, the reduction of sperm motility in mice with treated of piper, decrease sperm count treated with parathion (Sobarzo and Obregn, 2000), nitrofurazone (Singh and Chakravarty, 2001), textile waste water (Suryavathi et al, 2005) reported the reduction in sperm count and motility. Toxicity of silk dye waste on Swiss albino male mice of different histopathological section as stomach and intestine, lung, testis, liver and mitigation using *M. oleifera* leaf extract (Serina et al, 2017).

Moringa oleifera has gained popularity as a life-saving nutritional power plant. The effect of *M. oleifera* leaf extracts recovers histological section of testis and sperm profile. The *M. oleifera* leaf extract has positive effect on spermatogenesis in rats and another animal. These results may be due to presence of flavonoids. Flavonoids are well known antioxidants that can ameliorate oxidative stress-related testicular impairments in animal tissues (El-Missiry, 1999; Ghose et al, 2002; Kujo, 2004). It also stimulates testicular androgenesis and is essential for testicular differentiation, integrity and steroidogenic functions (Dawson et al, 1990; Luck, 1995; Salem et al, 2001). This histoarchitectural evidence was the clear indication of confirming the Spermatogenic efficacy of extracts of *M. oleifera* leaves in male albino rats. The process of spermatogenesis and accessory reproductive organ function are androgen dependant. Similar finding 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 that were treated with *Moringa oleifera* after alcohol administration however, showed a largely preserved testis weights, testis weight/ body weight ratio and testis volumes (Ismail et al, 2007). Thus it can be concluded that *Moringa oleifera* impairs the morphology and functions of the testis in adult male mice. It is, therefore, advised that individuals consume *Moringa oleifera*'s products in moderate quantities while concerted

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efforts should be made by Nigerian scientists (2013) to determine experimentally the required doses of the different parts of *Moringa oleifera* for safe nutritional and medicinal purposes.

CONCLUSION

This study concludes that silk dye induced any alteration in histopathological section of testis and sperm parameters can be mitigated by the application of *M. oleifera*. On the basis of above discussed data and facts it can be concluded that the *M. oleifera* leaf powder significantly reduce the alteration arisen in reproductive ability and associated histological structures in the toxicity impact of silk dyes waste effluent induced male mice.

REFERENCES

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.

Chakravarty, Singh et al (2005): Toxicity of Industrial waste water to the aquatic plant Lemna minor. Environ. Biol; 27(20): 385-390.

Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life Journal.

Jahn, S.A.A. (1988): Using *Moringa oleifera* seeds as coagulant in developing countries. J Amer Water Works Assoc, 6:43-50.

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): 1-7.

Khatun, S. and Varma, M.C. (2017): Toxicity impact of silk dye waste effluent on reproductive system of swiss albino male mice *Mus musculus* and mitigation of *M. oleifera* leaf extract. World. J. of Pharmacy and Pharmaceutic Sciences, 6(1):1621-1629.

Kumar, N.A and Pari, L (2003): Antioxidant Action of *Moringa oleifera* Lam. against peroxidation in rats. J Med Food, 6(3):255-259.

Kumar, P (2011): Study on hypocholestemia in blood due to carpet dye. J. Haem and Ecotorial, 5(3):33-37.

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.

Mathur, N; Mehta, M; Chaudhry, V (2005): Sperm abnormality Induction by Food colour metanil yellow. J. Ecolphysiol. Occup. Hlth; 5:157-160.

Meyer, U. (1981): Biodegradation of synthetic organic colorants. FEMS Symp, 12:371-385.

Miller, K. (1982): Sensitivity to tartrazine. Br.Med.J; 285:1557-1558.

Mishra, R. K; Singh, S.K (2007): effect of aqueous leaf extract of *A. indica* on the reproductive organs in male mice. Ind. Jol. Of Exptl. Biol. 43:1093-1103.

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.

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

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.

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.

Serina, K. (2017): Toxicity impact of Silk dye waste effluent induced Histopathological changes in the Stomach and Intestine of Swiss albino mice *Mus musculus* and their mitigation using *Moringa oleifera* leaf powder. J. of Med. Sci. and Clin. Res, 5(3): 19306-19313.

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.

Serina, K. and Varma, M. C. (2016): Effect of *M. oleifera* leaf extract on silk dye effluent affected haematological and biochemical parameter on Swiss albino male mice. Amer. Inter. J. Form, Appli and Natu. Sci, 14(1), pp.50-55.

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.

Vaclav, I; Bruno, L (1993): Infertility of male and female, 2nd d, UK. London. Charchill Livingstone, 739.

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078