EFFECT OF SILK DYE WASTE ON REPRODUCTIVE EFFICIENCY OF MALE SWISS ALBINO MICE

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Abstract: Different chemicals and pollutants such as distillery wastes, industrial wastes, textile dye wastes have shown different toxic effects on different organisms. Silk dye waste is one among them. Aim of present investigation was to find out whether any changes in reproductive efficiency like fructose estimation, sperm profile (sperm head morphology, sperm count and sperm motility) and litter count after administered of silk dye waste on laboratory animal. Sperm count parameters are sensitive to unfavourable condition. So it has been consider the probing extent of hazardness of silk dye waste that affects the reproductive efficiency.

Keywords : *Mus musculus*, toxic effects, Silk dye waste, sperm profile.

I. Introduction:

In India textile, dye, pulp and paper, distillery and metal industries are the main sources of aquatic pollution. Effluents from these industries drastically change the physico-chemical properties of agricultural soil and river water. (Singh and Singh 2005). The textile industry is the largest consumer of dye stuff, at nearly 80%. Discharge of dye bearing effluents into natural water bodies from textile, carpet and printing industries has created significant toxicity. About 500-1,000 cottage silk weaving, dyeing and bleaching industry have been set up by the local people in Bhagalpur. Silk dye effluents have been discharged directly without any treatment due to technological and economical limitations. In mammals, the whole organism is committed to the process of reproduction and general well being is essential for reproductive efficiency. However, it has to be noted that effects on reproduction can predominate and or occur at exposures considerably lower than those causing other forms of toxicity. Present studies have reseated interference of toxicity in human reproduction (IARC, 1979).

II. Materials and Method:

Experimental animals: Experiments were performed with 3months old healthy laboratory–inbred Swiss albino mice *(Mus musculus).* Healthy albino mice weighing 25±5g obtained from CDRI, Lucknow and maintained in the animal house of University Department of Zoology, Bhagalpur, Bihar. All the animals were kept as accepted principles for laboratory animal use and care as per the guidelines of CPCSEA. The mice were acclimatized one week before the experiment. Experimental mice were kept in clean cages and fed on balance diet and distilled water to them was made available ad libitum for drinking purpose. Rice husk were used as bedding material and were changed daily. The animals were separated into two major groups-controls and treated ones.

Experimental dose and design: For studying the toxicity impact mice were divided into 3 major groups each group having 10 mice. The group I (control group) received 1ml distilled water. The treated groups (II & III) were received 1ml different concentration of dyeing waste. Treatments were continued for 15, 30, 45 and 60 days for biochemical (fructose), sperm profile and reproductive ability. Fructose estimation was done after the method of Mann (1964). Sperm Count methodology was followed as suggested by Rastogi and Levin (1987). For reproductive ability male rats were introduced to parous females,(male: female ratio, 1:3) at 24:00 h after 15 days, 30 days, 45 days and60 days of treatment. The successful mating was confirmed in the forthcoming morning's days by vaginal plug. The inseminated females were separated and allowed to deliver at term. The number of pups delivered and their characteristics were noted.

III. Results:

Fructose content of seminal vesicle of the Group I animals showed 91.09 ± 0.94 µg/g to 93.0.72±0.72 µg/g. Maximum decrease 24.87±1.25 µg/g was recorded in group III at 60 days exposure period. About 400 sperm from each experimental group of mice were screened randomly. A typical normal sperm with its characteristic shape is shown in plate 1. Abnormal head morphology Sperm showing different types of abnormalities in their head morphology (head shape, head size and head spine). In Group I, II and III of animals, almost all types of head abnormalities were recorded (Plate 1.1-1.2). In group I their frequencies were very low (Table 1).The total number of abnormalities among group I of mice were 83 (4.15±1.04) to 119(5.95±1.09) in which sperm head shape (Amorphous, Pin head, Banana, Dumb-bell, Broad base, club, Hammer head) were 73(3.65±1.08) to92(4.60±0.61), head number abnormalities were 0(0.00±0.00) to 4 (0.2±1.00) and head spine were10(0.5±4.50) 263(13.65±1.84) were recorded in group III mice treated for 60 days. Sperm count of group I showed (16.17±0.16)×10^4/ml, (16.34±0.16)×10^4/ml, (16.48±0.16)×10^4/ml and (16.57±0.12) ×10^4/ml with different time duration.

Group II showed variation in total sperm abnormalities 121(6.05±0.97), 151(7.55±1.32), 165(8.25±1.77) and 172(8.60±1.84) after 15 days, 30 days, 45 days and 60 days respectively. Maximum variation recorded in Group III animals (10.43±0.19)×10^4/ml, (9.44±0.10)×10^4/ml, (6.07±0.18)×10^4/ml and (4.19±0.08) ×10^4/ml (Table 3). Sperm Motility of group I animal showed (81.09±0.20). Table 1, 2, 3 & 4 showed that the impact of different concentrations of silk dye waste on fructose, sperm profile were significant at 5% level. Litter count of group I animal showed 12.2±0.49 to 11.7±0.33. After long term exposure (15 days, 30 days, 45 days and 60 days) the treated group II showed 10.8±0.46, 10±0.36, 9.7±0.0.30, 6.8±0.29.

Table 1: Fructose content observations of male mice after different exposure period in control and treated with different concentrations of silk dye waste

Experimental Group	Exposure Period			
	15 days	30 days	45 days	60 days
Group I	91.09±0.94	93.78±0.91	94.75±1.21	97.32±0.72
Group II	55.09±1.27	49.96±0.99	48.65±0.30	47.18±1.04
Group III	37.24±1.02	28.70±1.13	25.42±0.92	24.87±1.25

Table: 2 Frequency of total abnormal sperms in control and dye waste treated mice Groups with different concentrations after different exposure period

	Group I				Group II				Group III			
Types	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
Head Shape	3.65± 1.08	3.9± 2.17	4.25± 3.44	4.6± 4.65	4.65± 0.87	6.1± 2.02	5.8± 3.41	7.1± 4.63	5.45± 0.73	7.40± 1.85	9.55± 2.94	11.45± 3.99
Head No	0.1± 0.00	0.20± 0.00	0.25± 0.50	0.27± 1.00	0.7± 0.00	1.1± 3.15	1.9± 1.00	2.3± 1.00	0.95± 0.50	1.85± 3.50	2.7± 2.00	3.50± 3.50
Head Spine	4.15± 1.04	4,80± 0.90	5.50± 0.95	5.95± 1.09	6.05± 0.97	7.55± 1.32	8.25± 1.77	8.60± 1.84	7.85± 1.52	9.85± 1.90	11.75± 1.80	13.15± 1.84

Table 3 : Sperm count observations of male mice after different exposure period in control and treated with different concentrations of silk dye waste

Experimental Group	Exposure Period			
	15 days	30 days	45 days	60 days
Group I	16.17±0.16	16.34±0. 16	16.48±0. 16	16.57±0.12
Group II	12.91±0. 16	11.43±0. 13	8.53±0.28	5.48±0.14
Group III	10.43±0. 19	7.44±0.10	6.07±0.18	4.19±0.08

Table 4 : Sperm Motility of male mice after different exposure period in control and treated with different concentrations of silk dye waste

Experimental Group	Exposure Period			
	15 days	30 days	45 days	60 days
Group I	81.09±0.20	81.43±0.18	81.83±0.17	81.88±0.12
Group II	65.33±0.27	63.58±0.48	56.16±0.14	37.80±0.57
Group III	62.17±0.41	50.26±1.31	46.16±0.44	26.57±0.95

Table 5 : Litter count of male mice after different exposure period in control and treated with different concentrations of silk dye waste

Experimental Group	Exposure Period			
	15 days	30 days	45 days	60 days
Group I	12.2±0.49	11.9±0.37	11.7±0.34	11.7±0.33
Group II	10.8±0.46	10±0.36	9.7±0.30	6.8±0.29
Group III	9.7±0.42	7.8±0.32	5.8±0.35	3.00±0.33

IV. Discussion

Fructose correlated positively with volume and head defect and negatively with sperm count (Gonzales, 1997). The concentrations of fructose in seminal plasma justify further studies into their dynamics *Vis a Vis* male infertility. Additionally, seminal plasma fructose concentration determination is useful for auxiliary diagnosis of obstructive and non-obstructive azoospermia. Seminal fructose concentration in non-obstructive azoospermia is usually higher than or equal to that in males of normal fertility (Buckett & Lewis, 2002). Sperm morphology of the mice were used as sensitive indicator for estimation of reproductive toxicity induced by silk dye after various incubation period (15 days, 30 days 45 days and 60 days) in treated groups .Study examined the effects of silk dye waste from a small cottage silk dyeing industries on sperm morphology of albino mice. 2000 sperms head were screened per duration per concentration of treated mice out of which the number and percentage of total abnormalities in sperm head shape, head size and head number were more in treated group II & III in comparision to group I.

Induced sperm abnormalities were also studied by other workers (Fiskesjo, 1988; Odeigah *et al*., 1997; Seetharaman *et al*., 2004; Babatunde and Bakare, 2006; Bakare and Wale-Adeyemo, 2004, 2009; Olorunfemi *et al*., 2011) have shown to be useful signs of cytotoxicity and genotoxicity. Staykova *et al*., (2005) have established the genotoxic and mutagenic effects of open water contaminated with heavy metals and cyanide, further confirming the results of the inhibitory effects of these s in previous studies (Olorunfemi *et al*., 2007, 2008, 2011). Presence of abnormal sperm head suggests induction of genetic damage in the male germ cells. Presence of abnormal sperm head suggests induction of genetic damage in the male germ cells. Sperm head abnormalities may arise due to small deletions or point mutations. Abnormalities in sperm head may occur by physiological, cytotoxic or genetic mechanisms, alteration in testicular DNA which in turn disrupts the process of differentiation of spermatozoa (Odeigha, 1997). The formation of normal sperm head involves a series of intricate, synchronous, morphological and biochemical steps. The nuclei that result from these processes are normally very

Plate : Abnormalities in Sperm Head Morphology

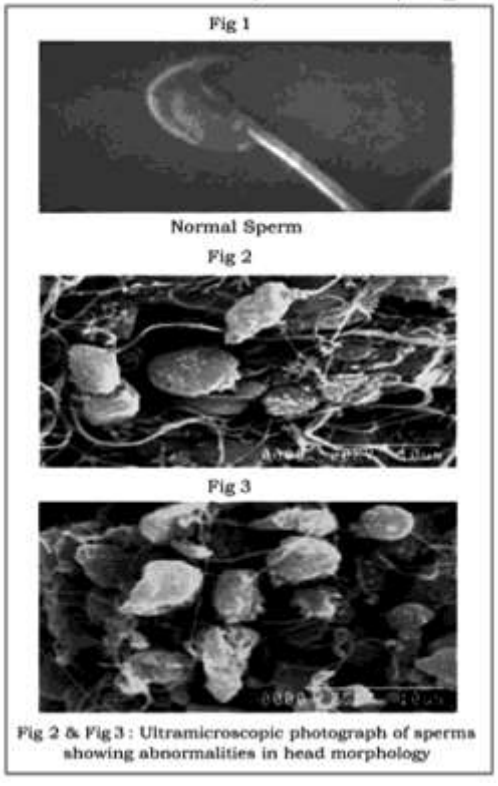

Fig 2 & Fig 3 : Ultramicroscopic photograph of sperms showing abnormalities in head morphology

homogenous and have a marked strain specific structural definition (Beatty, 1970). Chromosomes appear to be arranged in specific location in the nuclei (Beatty, 1970). The reason for sperm of abnormal head shape is perhaps they are the results of naturally occurring levels of mistakes in the differentiation process. Genotoxic chemicals might increase the frequency of these mistakes. If this were the case, then sperm with abnormal head shapes might well contain an intact, normal chromosome complement or perhaps there are very few mistakes made in the packaging of genetic material in the head. Perhaps, the abnormal shape is a consequence of an abnormal chromosome complement.In the present investigation after long term exposure to different concentrations of silk dye has shown reduction in sperm count and motility (Table 3 and 4). Sharma S. et al. (2007) reported similarin albino rat treated with textile dye wastewater. The reduction in sperm count and motility after silk dye treatment was due to degeneration in spermatogenesis process, epididymis and seminal vesicle the accessory organ. The potential adverse effects of erythrosine on the sperm profile were investigated in adult mice. Sperm count as well as the percentage of motile sperms were significantly decreased by about 50% and 57% erythrosine administration respectively (Abdel Aziz, 1997). Suryapathi *et.al.* (2005) reported reduction in sperm counts (10-59%) and motility (14-56%) with higher sperm abnormalities after histopathological studies of textile dye wastewater treated male mice and rats due to altered spermatogenesis. The reduction in the sperm density observed in the experimental animals might be attributed to the altered androgen metabolism due to dye toxicity (Choudhary, 2005;). Silk dye waste has shown to disrupt the normal morphology of the sperm head. After administrations of silk dye waste at 60 days it increased the incidence of sperms with abnormal head by about group II and III respectively. The induced increase in sperm abnormalities could enhance the spermatogenic dysfunction and germ cell mutagenicity. These findings indicate that silk dye waste in the used doses has a potential toxic effect on spermatogenesis in mice and in turn, it may affect its testicular function and reproductive performance.

Silk dyes are considered to have potentials for endocrine disruption (Jenkins *et.al.*, 2001).Androstanedione, phytoestrogens and other compounds that can interact with steroid receptors are suggested to interfere with the normal functioning of the endocrine system, thus affecting reproduction and development in animals (Parks *et.al.*, 2001; Durhan *et.al.*, 2002). In addition, judging from the response of the various segments of the male reproductive tract it appears that the testis is more sensitive and responsive to the treatment than the other accessory organs. Male mice and rats were exposed to MIX in the drinking water at concentrations of 1, 5, and 10% the cauda epididymal sperm concentration and sperm count were shown reduced (20%). Prenatal exposure to fetal germ cells toxicants permanently reduced sperm production in male progeny (Gray, Jr LE. 1996). Azo dyes exhibited in some strains of rats and mice, production of sperm can be reduced by 90% or more (Francis et al., 1994).Toxic substances may interfere with the production of sperm resulting in adverse effect in number and morphology of sperm (WHO 1999).The amino azo toludine containing dyeing in sperm morphology of mammal has been associated with abnormal spermatogenesis. Graca *et.al.* (2004) has also reported lead treated mice significantly ($P < 0.05$) lower epididymal sperm density and increase percentage of immotile sperm. Sharma S. *et.al.* (2011) observed significantly decrease in sperm count and motility in mice when treated with distillery soil lacheate. Serious toxic effect on the sperm morphology and sperm count in mice after administration of Metanil yellow reported by Mathur *et.al.* (2005). These findings are consistent with the studies of Wyrobek *et.al.*, (1983); Krishnamoorthy and Laheri (2001); Mathur *et.al.* (2005). Several kind of mutation can lead to abnormal sperm morphology. The abnormal sperm shape can be caused by protein abnormality, as sperm shape is partially imparted by structural protein (Wyrobek *et.al.*, 1983). Reduction in the sperm count and motility, reduced fertilizing ability which might be due to decrease in total protein content in cauda epididymis (Verma and Chinoy, 2001). Similar results were observed by (Suryavathi *et.al.* 2005, Sharma *et.al.* 2007) in rats after treated with textile dye .s Marked reduction in sperm count cause infertility. Sperm motility is one of the most important parameters used in the evaluation of sperm quality. Sperm motility is also an important functional measurement to predict sperm fertilizing capacity (Barratt *et.al.*, 1993).

Conclusion

The results suggest that increase in head morphology abnormalities ,reduction in the sperm count and motility of albino mice could be as a result of heavy metals interaction of waste coming out the from silk dyeing industries. The toxic effects of the waste from silk dyeing industries established in this study indicates that the waste contain toxic substances which may constitute a risk to the environment and human health, more especially as the waste generated from silk dyeing processing is not properly managed.

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