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Research Article

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

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A B S T R A C T

The object of this histopathological study was to investigate critical lung toxicity caused by silk dye waste effluent and amelioration by using of medicinal plant *Moringa oleifera* leaf extract.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 histological examinations of this study revealed a damage and degeneration in the lung tissues of the silk dye treated animals. *Moringa oleifera* administration to silk dye treated mice showed reduction in the tissue damage and amelioration of restored the normal distribution of elastic fibres in lung. These results, along with previous observation, suggest that *M. oleifera* may be useful in lung tissues injury that is a result of silk dye waste effluent toxicity.

INTRODUCTION

The human are exposed to various types 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 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).

Moringa oleifera is considered to be an important medicinal plant. It is commonly known as 'drumstick' and is being used as antiulcer, 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).

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But no work has been done on its property to mitigate the damages induced by silk dye waste on histopathology of lung of Swiss albino mice. Hence the present work has been undertaken to study the toxicity impact of silk dye waste on lung of albino mice and their subsequent regaining by application of *Moringa* leaf powder.

This study was therefore designed to investigation the effect of *Moringa oleifera* on silk dye waste effluent induced lung in male mice *Mus musculus*.

MATERIALS AND METHOD

Animals: Experiment was performed on 6 to 8 weeks old healthy laboratory inbred male *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 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 on lung.

Tissue processing and staining: After 30 and 60 days of experiment, mice were sacrificed and their organs were removed, were fixed in fixative and paraffinised, Haematoxylin-Eosin stained sections of lung were observed under light microscope (Pears, 1985) on 40X magnification.

Group I (Fig 1)

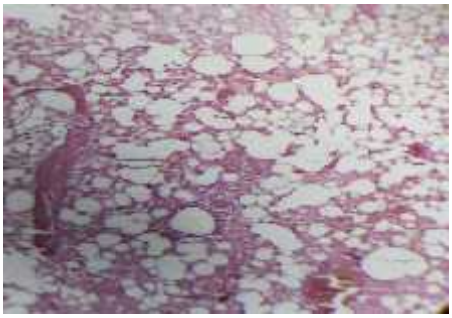


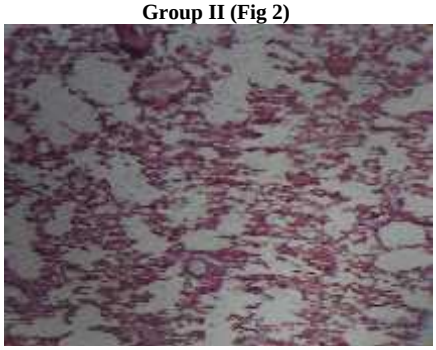
Figure 1 Photomicrograph of lung section of mice showed normal histoarchitecture. (x40, H&E)

RESULTS

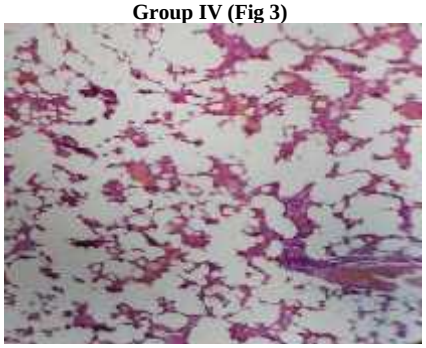
Histopathological observations on Lung: Lung sections of Group-I animals showed normal lung architecture with thin interalveolar septa and clear alveoli, alveolar sacs and normal alveolar septa with regular air sacs. The interalveolars spaces may be extremely narrow (Figure-1).

Group-II treated with 50% silk dye waste effluent at 30 days showed variable histological changes in which the alveolar septa appeared increased in thickening with mild inflammation compared with control group (Figure-2). Lung section of the Group-IV mice treated with *M. oleifera* leaf extract showed some amelioration in its architecture. This was manifested by almost normal appearance of most air sacs and interalveolar septa due to decrease in thickening of interalveolar septa and most of air sac returned to normal shape and size (Figure-3). Group-III treated with 100% silk dye waste at 30 day incubation period showing extensive destruction of their walls resulting in the formation of enlarged, irregular air space and the architecture of the lung was not preserved (Figure-4). Lung section of Group-V treated with *M. oleifera* leaf extract showedmore amelioration in its architecture with more or less normal interalveolar septa and normal appearance of air sacs (Figure-5).Also, dilated congested blood vessels were detected in interalveolar septa. Group-II treated with 50% silk dye waste at 60 days showed, the thickening of septa and irregularity and enlargement of air sacs were increased. In addition, also marked dilated congested blood vessels and inflammatory cells infiltration were prominent features observed (Figure-6).At 60 days *M. oleifera* leaf extract treated mice (Group-IV) lung section showed significant mitigation in its architecture, normal interalveolar septa and reduction of focal inflammatory cells, normal appearance of air sacs but still regular in shape than normal (Figure-7).

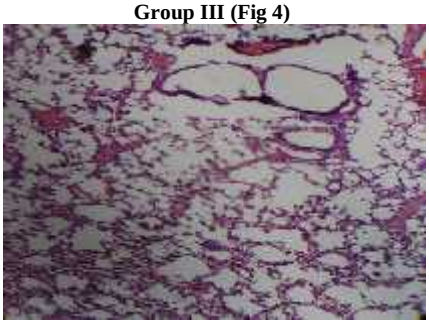
Group II (Fig 2)



Group IV (Fig 3)



Group III (Fig 4)



Group V (Fig 5)

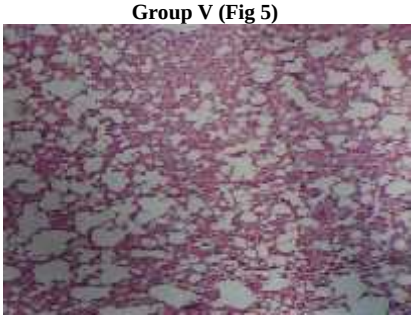
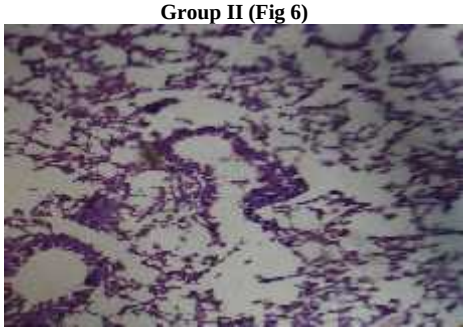


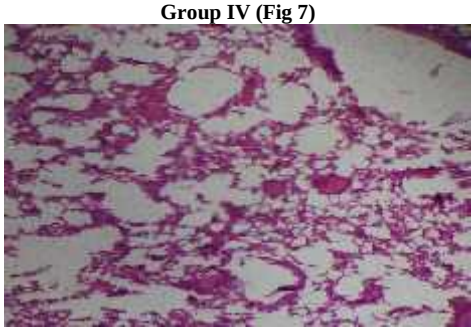
Figure 2 Photomicrograph of lung section of mice showed, mild inflammation.(x40, H&E).Figure:-3. Photomicrograph of lung section of mice showed, normal shape and size of interalveolar septa and air sacs. (x40, H&E).Figure:-4. Photomicrograph of lung section of mice showed, enlarged septa wall and irregular air space.(x40, H&E).Figure:-5. Photomicrograph of lung section of mice showed, normal interalveolar septa and normal appearance of air sacs. (x40, H&E).

Group-III treated with 100% silk dye waste effluent at 60 days showed, disappearance of the alveolar septa in most areas, increased irregularity and size of air sacs and destruction of normal tissue pattern, inflammatory cells observed could be seen (Figure-8). Group-V histological observation of lung section of mice treated with *M. oleifera* leaf extract revealed normal lung architecture, thin septa and regular air spaces and nearly similar to the control. Significant recovered of inflammatory cells in interalveolar septa (Figure-9).

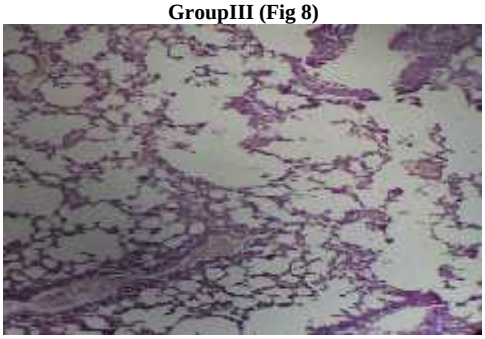
Group II (Fig 6)



Group IV (Fig 7)



GroupIII (Fig 8)



Group V (Fig 9)

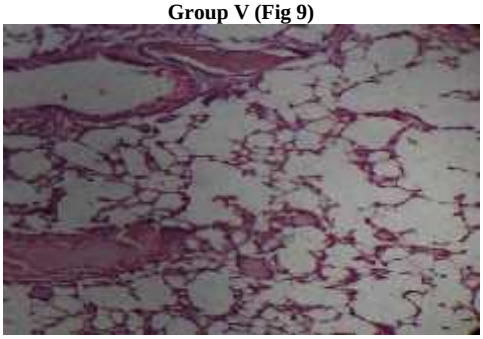


Figure 6 Photomicrograph of lung section of mice showed, marked dilated congested blood vessels and inflammatory cells infiltration. (x40, H&E). **Figure 7** Photomicrograph of lung section of mice showed, normal interalveolar septa, focal inflammatory cells, normal appearance of air sacs and still irregular in shape than normal. (x40, H&E). **Figure 8** Photomicrograph of lung section of mice showed, disappearance of the alveolar septa in most areas, increased irregularity and size of air sacs, inflammatory cells. (x40, H&E). **Figure 9** Photomicrograph of lung section of mice showed, normal lung architecture, thin septa and regular air spaces, recovered of inflammatory cells in interalveolar septa. (x40, H&E).

DISCUSSION

In the present experimental study were investigated, the histopathological effects of silk dye waste effluent on mice lung and their significant mitigation using *Moringa oleifera* leaf extract. In this study, the histopathological examinations of lung tissue of mice treated with silk dye waste throughout the experimental intervals revealed variable gradations of alteration. In this study, a destruction of alveolar tissue or alveolar walls and formation of enlarged, irregular air sac may be attributed to chronic inflammation caused by nicotine administration (Piipari *et al*, 2000; Park *et al*, 1998). Trombino *et al* (2004) and Luqman and Rizvi (2006) showed presence of interstitial inflammatory cells, degenerative changes in the interstitial cells and alveolar epithelial cells caused by nicotine. The alteration in pulmonary structure was improved by previous studies (Valenca *et al*, 2004; Demiralay *et al*, 2006) which suggest that few septa fragments were thickened, many enlarged airspaces were detected, accompanied by destruction of alveolar septa.However, intratracheal instillation is an easies method than inhalation and has been proposed as a reliable route for assessing the pulmonary toxicity of particles in rodents (Warheit *et al*, 2005; Yokohire *et al*, 2008). In addition, similar histopathological results have been previously observed for both methods (Warheit *et al*, 2005). Activation of the anti-inflammatory cholinergic pathway, either vagal stimulation (Borovikova *et al*, 2000; Ochani *et al*, 2008) or direct activation of nicotinic acetylcholine receptors by nicotine administration (Mabley *et al*, 2002;Gwilt *et al*, 2007; Lee *et al*, 2007) has been demonstrated to be anti-inflammatory in a wide variety of inflammatory disease state (Tracey *et al*, 2007).

Moringa oleifera leaves provide the reasons for the need to establish its safety and toxicological profile. Alanine and Aspartate Transaminases are primary enzymes of the liver but are also present in the kidney (Nwangwu Spencer *et al*., 2011, Nwagwa, 2012). Elevated levels of Alanine and Aspartate Transaminases are, therefore, possible indicators of liver and kidney damage (Nwangwu Spencer *et al*, 2011; Nwagwa, 2012). Similarly, urea concentrations provide one of the direct measurements of glomerular filtration rate and when elevated is indicative of kidney damage (Nwangwu Spencer *et al*, 2011; Nwagwa, 2012). The amelioration effect of green tea on nicotine toxicity may be attributed to antioxidant and anti-inflammatory properties (Varilek *et al*, 2001; Patra *et al*, 2008) and the free radicals scavenging properties (Neogy *et al*, 2008) through decreased lipid peroxidation and suppressed oxidative damage; both caused oxidative damage in nicotine treatment animals (Ogura *et al*, 2008). Knekt *et al* (2005) obtained that the lung tissue injury can be caused by nicotine toxicity and protection of tissues against reactive oxygen species caused by green tea.

In addition, *Moringa oleifera* leaf extract have mitigated reduction in elastic fiber caused by toxicity of silk dye waste.

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