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Role of *Moringa oleifera* leaf extract on silk dye waste effluent inducedhistopathotoxicity on liver and testis of Swiss albino male mice *Mus musculus.*

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Abstract: This work focuses primarily on the effects of *Moringa oleifera*leaf extract on histopathology of silk dye effluent induced histopathotoxicity in swiss albino male mice *Mus musculus*. The histopathological parameters have been taken an account. 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 atrophy of germinal epithelial cells, basement membrane, and process of spermatogenesis in different stage in case of testis and in case of liver, enlargement of the sinusoidal space, vacuole formations in hepatocyte, infiltrations with haemorrhage in hepatic tissuebut 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 histopathological constituents such as Liver and testis.

Keywords:*Moringa oleifera leaf powder, Silk dye waste effluent, histopathology,liver, testis, Swiss albino male mice.*

I. 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 godsend 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'Elongitude) 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 one of the world's most useful trees, as almost every part of the tree can be used for food, or has some other beneficial medicinal properties. It is commonly known as 'drumstick' and is being used as antiulcer, diuretic, anti- inflammatory, anti-microbial, potent- antioxidant and wound healing agent (Caceres et al., 1991; Udupa et al., 1994; Kurma and Mishra, 1998;Saalu et al., 2011 Bassey et al., 2013), pharmacological properties (Oliveira et al, 1999), it's an exceptional nutritious vegetable (Ram,1994).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). But no work has been done on its property to mitigate the damages induced by silk dye waste on histopathological changes of Swiss albino mice. Hence the present work has been undertaken to study the toxicity impact of silk dye waste on different histopathological tissues of albino mice and their subsequent recuperating by application of Moringa leaf powder.

This study was therefore designed to investigate the effect of*M. oleifera* on toxicity impact of silk dye waste induced histopathological changes as liver and testisin Swiss albino male mice.

II. MATERIALS AND METHOD

Animals: Experimentwas 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 Nathnagar, Bhagalpur at regular interval.

Experimental Design: The mice were divided into 5 groups of 6 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 wastewas 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 assay: Histopathological observation as Liver and Testis.

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

III. RESULTS

Histopathological Observation on Testis: The control group of mice (Fig-1) showed normal histological architecture of the testis. Group-II treated with 50% silk dye waste effluent (Fig-2a) showed numerous atrophied and damaged seminiferous living cells, spermatogoniaand spermatocyte.Group-IV treated with M. oleifera leaves powder at 30 days mitigated to damaged Spermatogenic living cells, spermatogonia, spermatocyte, spermatids and spermatozoa (Fig-2b).Group-III treated with 100% silk dye (Fig-2c) showed numerous atrophied and damaged seminiferous living cells, spermatogonia, spermatocyte, spermatids, spermatozoa, damage of basement membrane, lumen filled with semen, interstitial leydig cells and interstitium against background of connective tissues with marked area of necrosis. Group-IV treated with M. oleifera leaves powder at 30 days no more recovered of damaged basement membrane but slightly mitigated Spermatogenic living cells, spermatogonia, spermatocyte, spermatids and spermatozoa, interstitium against background of connective tissues with slight area of cellular alteration (Fig-2d).Group-II (treated with 50% silk dye effluent at 60 day) histological section of testis showed completely damaged basement membrane of seminiferous tubules with few spermatogonia and spermatocyte cell, disrupted of connective tissue, no spermatozoa having found (Fig-3a). Group-IV treated with M. oleifera leaves powder after 60 day incubation period showed recovery of damaged basemen membrane, seminiferous tubule, appeared connective tissues, sertoli cell, clear vision of different stage of spermatogenesis as spermatogonia, spermatocyte, spermatid and spermatozoa (Fig-3b). Group-III treated with 100% silk dye at 60 days incubation period, showed completely destroyedseminiferous tubule and Spermatogenic stage, connective tissue with marker area of necrosis (Fig-3c). Group-V treated with M. oleifera leaves powder after 60 days incubation period histological section of testis (Fig-3d) showed almost normal histoarchitecture as spermatogonia, spermatocyte, spermatids, spermatozoa and connective tissues which are comparable to the control group.

Group-I (Control Testis, Fig-1)

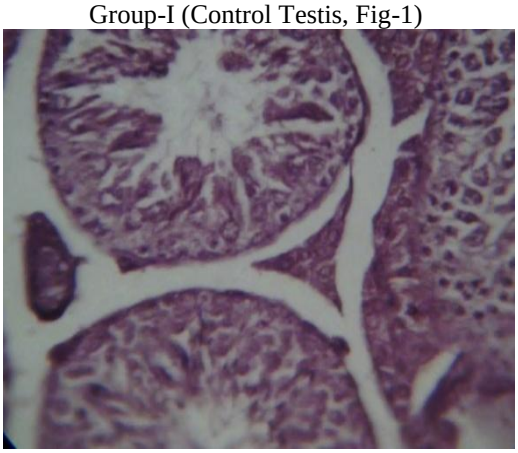

Figure:-1. Photograph showing the control groups liver of mice. (H&E, x40).

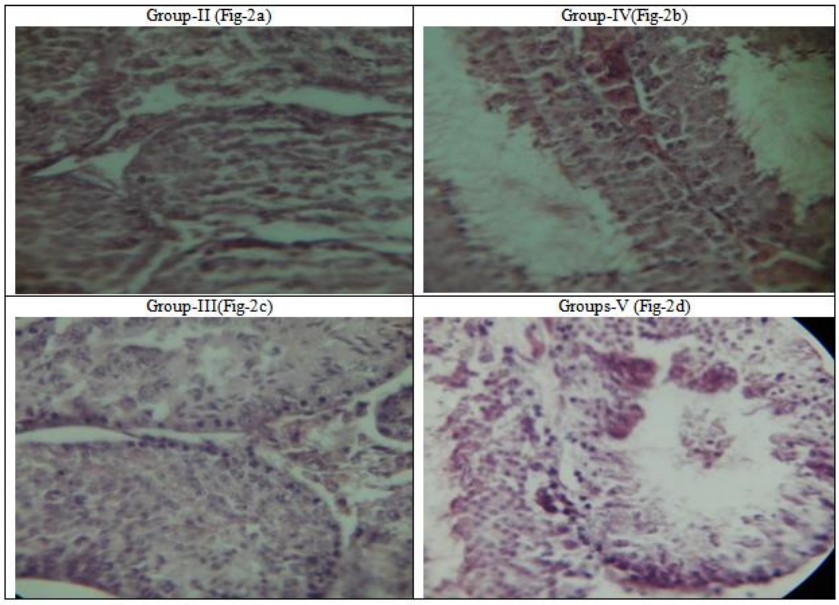

Figure: -2a-Group-II (atrophy of spermatogonial cell), **2b**-Group-IV (mitigation of spermatogenic cells), **2c**-Group-III (atrophy of spermatocytes, spermatids and spermatozoa), **2d**-Group-V (amelioration of spermatocyte, spermatogonial cells).(H&E, x40).

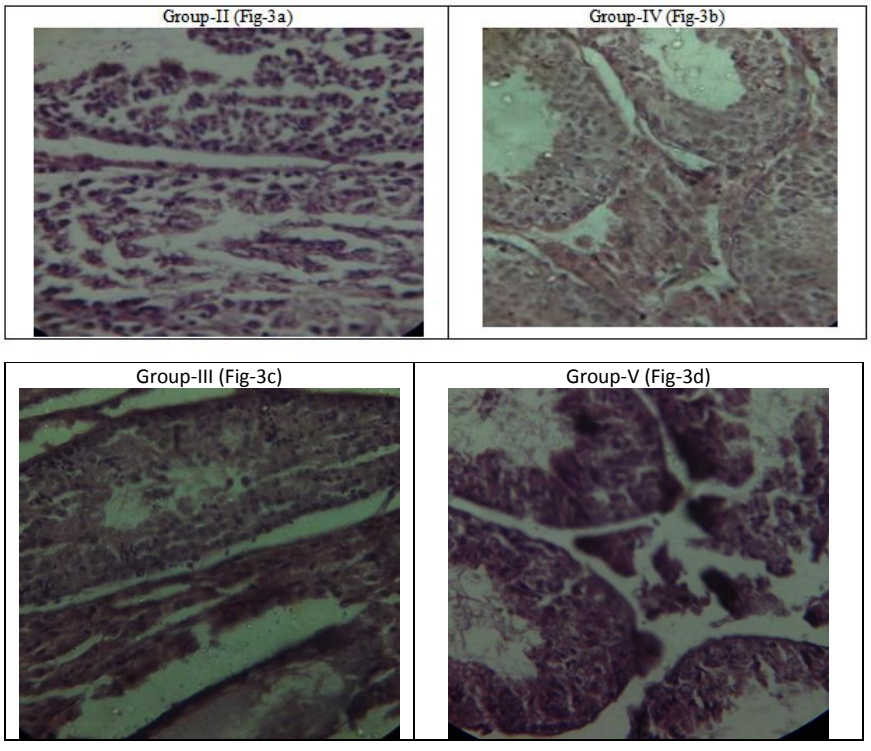

Figure: - **3a**-Group-II (Damage of spermatocyte and spermatids), **3b**-Group-IV (Present of spermatogonial cell, spermatocyte, spermatozoa and spermatids),**3c**-Group-III (completely damage testis), and **3d**-Group-V (revealed normal testis architecture).(H&E, x40).

Histopathological Observation on Liver: Examination of H&E section of the liver of the control group showed normal liver structure (Fig-4). At 30 days of treated with 50% and 100% silk dye waste effluent, the liver section appeared with mild vacuolization, slight enlargement of their nuclei. This also showed mild number of Kupffer cells, many vacuoles were observed in most of the hepatocytes. Also, the nucleus-cytoplasmic ratio was changed and the hepatic sinusoids narrowed in some areas compared to Gr-I (Fig-5a and 5c).After 60 days

of treatment, the liver section appeared with variable changes and marked injury. These changes were evidenced by disruption of tissue architecture, and sever vacuoles degeneration in the majority of hepatocytes. Also, the blood sinusoids disappeared in most areas of hepatic tissue and increased Kupffer cells compared to Gr-I of 50% and 100% silk dye waste effluent treated (Fig-6a and 6c).Treatment with Moringa oleifera leaf powder showed normal liver structure with normal apparent and decrease in hepatocytes vacuolization compared with silk dye waste effluent groups, at 30 day of treatment; the liver sections showed much mitigation in the liver tissue compared to Gr-II and III (Fig-5b and 5d). At the end of the experiment, M. oleifera reduced liver injury due to silk dye waste toxicity and largely suppressed activation of Kupffer cells and restored more or less normal size of nucleus and sinusoidal space. At 60days of treatment with M. oleifera leaves powder, similar architecture to the control group such as vesicular round nuclei with mild fatty degeneration or vaculation in focal area of hepatocytes was exhibited. The size of nucleus, number of nucleus, appeared compared to silk dye treated Gr-II and III at same stage of treatment (Fig-6b and 6d).

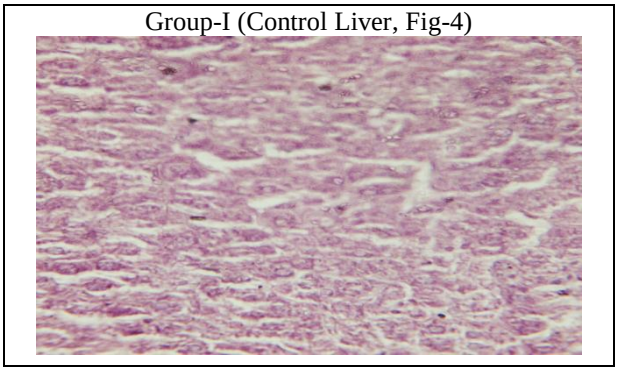

Figure:-4. Photograph showing the control groups liver of mice. (H&E, x40).

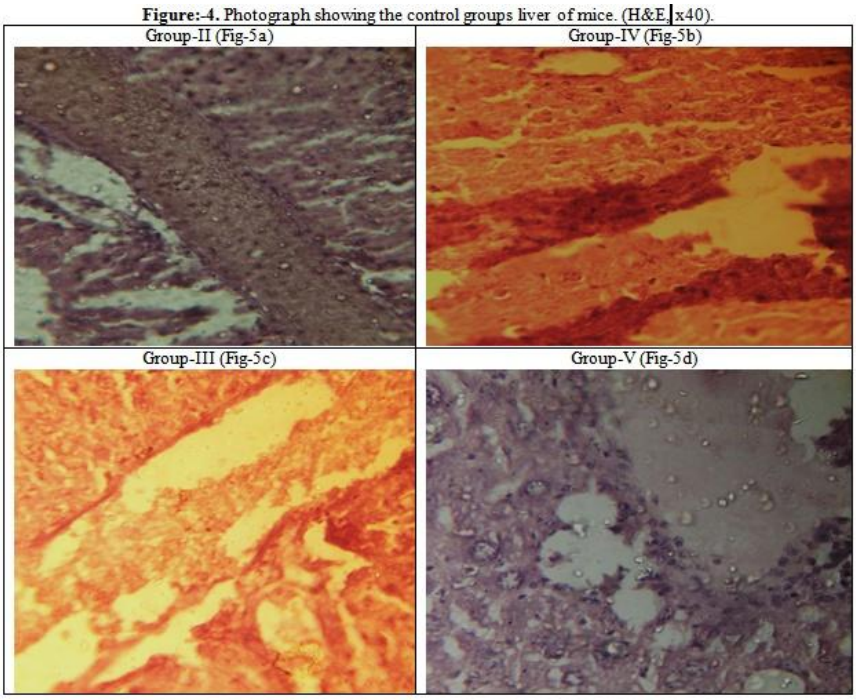

Figure: -5a.Group-II (mild vacuolization), **5b**.Group-IV (restored normal size of sinusoid),**5c.**Group-III (vacuolization in hepatocyte cells), and**5d**.Group-V (decrease vacuoles of hepatocyte cells).(H&E, x40).

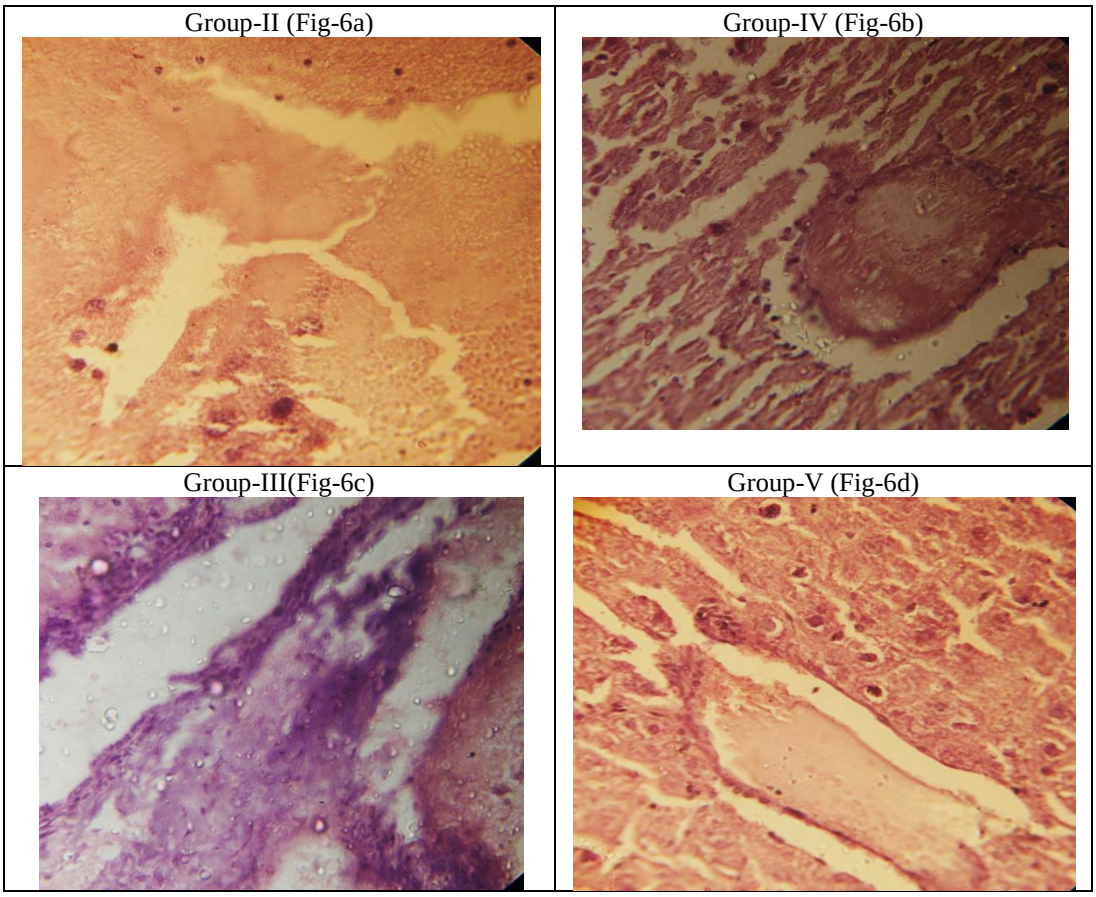

Figure: -6a-Gr-II (disruption of tissue architecture),**6b**-Gr-IV (amelioration of hepatocyte and number of nucleus),**6c**-Gr-III (blood sinusoid disappear) and **6d**-Gr-V (liver section indicated normal histology and architecture).(H&E, x40).

IV. DISCUSSION

The results show that the *M. oleifera* leaf extract when fed to Gr IV and V mice, showed the significant recovery of testis and liver 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. 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).This vitamin 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 in reproductive system (Khatun et al, 2017).

Histological observation of testicular sections of Pb treated mice reveals germ cell disorganization, epithelial vascuolization and cell loss as described by Batra et al, 1998. Additionally, the effect of environmental lead on the male reproductive system has been a major area of concern for several years by which the testicular spermatogenesis and spermatozoa within the epididymis are the major targets for lead action to produce toxicity on reproduction (Wadi and Ahmad, 1999). 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).

In the present study, the histopathological change in the liver of silk dye waste effluent treated mice suggest additional specific pathological pathway may be involved in silk dye hepatotoxicity and liver cell injury may be attributed to iron deposition in hepatocytes (El-Zayadi, 2006). The liver is well known target organ of the toxic impact regarding its function in biotransformation and excretion of xenobiotic (Roganovic and Jordanova, 1998). Selmanoglu and Akay (2000) who reported similar Histopathological changes including mononuclear cell infiltration, congestion and hepatocellular damage in the liver of male rats treated with dimethoate, endosulfan and carboxyl. (Sayim, 2007; Gokcimen et al, 2007; Ethalwagy et al, 2008 and Muthuviveganandave et al, 2011) suggested that may occur haemorrhage, inflammatory cell infiltration. The results from the present experiment are in agreement with the finding studied by Grewal KK et al, (2010).Additional specific pathogenic pathway may be involved in nicotine hepatotoxicity, as reported by El-Zayadi (2006), Yildiz (2004) and Muthukumaran et al. (2008), liver cell injury, inflammation and activation of Kupffer cells may be attributed to pro-inflammatory cytokines (El-Zayadi, 2006). These similar results are reported for Malathion and other pesticides indicating exposure of these pesticides leading to encourage histological disturbances in experimental animals (Ahmad et al, 2009; Yousef et al, 2003; Adeniran et al, 2006). On the other hand, M. oleifera attenuates histological damage by increasing the level of GSH (Yumei et al., 2006; Helieh and Theresa, 2008). Exposure to toxicity caused significant decrease in hepatic superoxide dismutase level (Muthukumaran et al, 2008; Sheng et al, 2001).The mitigation effect of green tee on nicotine toxicity may be attributed to anti-inflammatory and antioxidant properties (Varilek et al, 2001; Patra et al, 2008; Zhen et al, 2007; Chen et al, 2002) and the free radicals scavenging properties (Neogy et al, 2008) through decreased lipid peroxidation and suppressed oxidative damage; both caused oxidative damage in lead treatment animals (Ogura et al, 2008).Moreover, Gardner et al, (1925) and Lamson, Gardner, Gustafson, Maire, McLean and Wells (1924) have the effects of carbon tetrachloride depended on the mode of administration, a point also made by the post-administration of Wistar rats with leaf extract of Moringa oleifera remarkably modulated the oxidative stress caused by alcohol administration. This is potentials of Moringa oleifera had been demonstrated in our earlier study (Saalu et al, 2011).The reversal of elevated serum intracellular enzyme levels by M. oleifera extract after ethanol administration may be attributed to the stabilizing ability of the cell membrane preventing enzymes leakages as earlier postulated by Pari and Karthikesan (2007).

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