Pak. j. sci. ind. res. Ser. B: biol. sci. 2023 **66B**(3) 249-254

Ameliorative Effects of *Syzygium cumini* and *Morus alba* Fruit Extract on the Testicular Histopathology of Lead Exposed Mice

Muhammad Ali Kanwal[a], Amna Mehmood[a], Aneela Anjum[a], Iram Inayat[a], Syeda Nadia Ahmad[b], Sadia Suleman[a] and Saira Siddique[a] and Khawaja Raees Ahmad[a]*

[a]Department of Zoology, University of Sargodha, Sarodha, Pakistan
[b]Department of Zoology, University of Chakwal, Chakwal, Pakistan

(received April 12, 2021; revised October 16, 2021; accepted December 12, 2021)

Abstract. The present study focused the toxicity of lead (Pb) on the male mice reproductive system. The research was designed to check the histopathology of male albino mice testes after lead (Pb) exposure in drinking water and the rescuing effect of post treatment with Jambul Fruit Pulp Extract (JFE) and white Mulberry Fruit Pulp Extract (MFE). There were four groups and each group having 10 animals. Control group (no treatment), Pb group (50 ppm Pb ions in drinking water for 15 days) and for the next 5 days lead free water. Pb+J group (Pb treatment as in Pb group + 0.2 mL Jambul fruit pulp extract with 12 h intervals for 16-20 days of the study. Similarly, Pb+M group (Pb treatment as in Pb group + 0.2 mL Morus fruit pulp extract with 12h intervals for 16-20 days of the study. Results revealed various characteristic changes in testicular histology. The results indicated significant alternations in organ weight, number of spermatogonia, number of spermatocytes, cross sectional area (CSA) of seminiferous tubules, seminiferous epithelial height and the number of seminiferous tubules in Pb group than the control group. These results were significantly recovered in the Pb+J and Pb+M groups.

Keywords: lead, *Syzygium cumini*, *Morus alba*, testicular histopathology, mice

Introduction

Heavy metals cause environmental problems and lead poisoning is one of the global issues. Lead can enter in the body through intestines, skin or lungs (Gomes *et al*., 2015). It is commonly present in soil, air, water, paint and cosmetics (Kianoush *et al.*, 2015). Lead causes toxicity in several organs of humans and animals, mainly to the testes (Li *et al*., 2018). The reproductive system can be disrupted by heavy exposure to lead (Hu *et al*., 2019). Numerous studies have shown that Pb induces injury in testis of children and adults. It also disturbs the sperm quality and DNA integrity in mice (Li *et al*., 2018). The male reproductive system when exposed to different concentrations of lead resulted in alterations in spermatogenesis, sperm functional parameters and reproductive hormones (Santhosh and Asha, 2018), consequently affecting almost all aspects of reproduction (Latif, 2015). A semen analysis showed that too much reactive oxygen species (ROS) in semen upset sperm parameters, sperm concentration and motility (Takeshima *et al*., 2021).

Syzygium cumini (Jambul) is a perennial tropical tree belongs to the Myrtaceae family (Lanjewar *et al*., 2018). Various parts of the plant such as the bark, leaf, fruit and seed have been investigated for their bioactive phytochemical constituents (Chhikara *et al*., 2018). The fruit has numerous nutritive and bioactive compounds including fructose, malic acid, anthocyanins, tannins, gallic acid, glucose, citric acid, raffinose, petunidin, malvidin cyaniding and glycosides. Fruit possesses the valuable antibacterial, antioxidant and anti-inflammatory properties (Singh *et al.*, 2018).

Morus alba (white mulberry) is the dominant species among all 150 species of the Genus Morus (Mulberry) (Ustundag and Ozdogan , 2016). The fruit has a large number of nutritive compounds including amino acids, fatty acids, vitamins, minerals, anthocyanin, quercetin, chlorogenic acid and polysaccharides. Fruit extract has various biological activities including antioxidant, neuroprotective, antiatherosclerosis, antihyperglycemic, hypolipidemic and antitumor (Yuan and Zhao, 2017).

Materials and Methods

Animal rearing maintenance and feeding. Swiss Webster mice were used to carry out the research work in the animal house established in the Department of Zoology, University of Sargodha, Sargodha, Pakistan. The Animals were nurtured and sustained under the

*Author for correspondence; E-mail: raees.ahmad@uos.edu.pk

established protocols of our lab 12h dark-light cycles, 23±2 °C temperature with free access to food and water. The protocol was designed according to international guidelines for the use of animals. This study was approved by ethical committee of the University of Sargodha. For the experiment thirty adult male animals, weighing between 28-30 g and aged 3-4 months were used.

Dose groups. Fourty animals were divided into four different groups (10 animals in each group).

Control group (C). This group was maintained purely on lead free water for drinking purpose.

Lead group (Pb). These animals were provided with 50ppm lead acetate in drinking water for 15 days followed by 5 days water.

Lead + *Morus alba* fruit extract group (Pb+M). These animals were given 50 ppm lead acetate in drinking water for 15 days followed by 0.2mL *Morus alba* fruit extract treatment regularly at 12 h intervals for 5 days and regular drinking water.

Lead +*Syzygium cumini* fruit extract group (Pb+J). These animals were given 50 ppm lead acetate in drinking water for 15 days followed by 0.2 mL *Syzygium cumini* fruit extract treatment regularly at 12h intervals for 5 days and regular drinking water.

Dose was decided by checking LD 50 of toxicant that can be safely consumed by animal. The treatment length was 15 days for lead administration because damaging effect can occur in this period and time period of 5 days was enough for *Morus alba* to show its rehabilitative property. During the whole study lead free water (Khush Aab- PSQCA Licence No: CML/N-137/11- a product of University of Sargodha) was used.

Lead acetate dose preparation. For getting 50 ppm Pb ion solution, a $PbCH_3COO$ 1000 ppm stock solution was prepared by dissolving 1.56 g of $PbCH_3COO$ in 1L of water. The dose (50ppm) was obtained by adding 950 mL of water and 50mL of stock solution.

Preparation of fruit extracts. Fresh fruits (Mulberry and jambul) were purchased from local market. The pulp was removed and ground. The juice was obtained by grinding the fruit in an electric blender. Fibrous contents were removed by centrifugation. Only the liquid supernatant was used for animal treatments.

Organ recovery. The animals were dissected after cervical dislocation to remove testis on day 21st of the experiment. For gross morphological study and record, testis was weighed and photographed separately *in-situ*. All testes obtained were finally fixed in acidified formyl ethanol (100%) for further study.

Histological arrangements and observations. For paraffin embedding and dehydration each testis was immersed for in 50 and 70, 90% and absolute alcohol for 3-5 h in each solution and finally in xylene for 5-6 h. A rotary microtome (ERMA TOKYO 42) was used to obtain 5 µ thick serial transverse sections which were placed on albumenized glass microscope slides and stained by using Hematoxylin and Eosin stain. A trinocular compound microscope was used to observe the histopathological alterations associated with lead exposure.

Digital photography and histological parameters. The digital photographs of selected histological sections were obtained and processed in Corel DRAW11 for the adjustment of sharpness, colour and contrast and cropping in order to highlight the histological parameters. The morphometric data was created from the digital photomicrographs of the histological slices of testis. Mean body weight, mean organ weight, number of spermatogonia, number of seminiferous tubules, average cross-sectional area of seminiferous tubules and seminiferous epithelial height were measured. The cross sectional area was obtained by the using following formula.

$$CSA = (Length \times Width/4)\ \pi$$

Data analysis and statistical applications. ANOVA and Tukey's multiple range test were used to analyzed the histological and micrometric based data.

Results and Discussion

Histological and micrometric analysis. The micrometric ersults of testies among the groups as shown in Table 1. The histological sections in the control group shows rounded sections of seminiferous tubules embedded in very well developed interstitial tissue containing sertoli cells and blood vessels. All sections of seminiferous tubules contain basement membrane at the outer surface and it contains concentric whirls of spermatogonia followed by multiple whirls of successive generations of spermatocytes and spermatids, while the developing spermatozoa and spermatids have been found near the inner most portion embedded through

their heads in the cellular mass of the spermatids and spermatocytes logically present on and above the sertoli cells (Fig. 1; 1A, 1D and 2A1).

In the Pb treated group different signs of testicular pathology were seen that include death of the interstitial tissue leaving wide gaps between the adjacent sections of seminiferous tubules. The basement membranes were irregular, the spermatogonia were either absent or extremely scanty in number present on the inner side of basement membrane with wide gaps and in most of such cases signs of cellular vacuolations and apoptosis were very obvious. The mass of spermatocytes was losing the individual cellular identifications, nuclear and cytoplasm of the individual spermatocytes seems to be completely disrupted forming a thick halo of disrupted cellular mass around the central lumen of the seminiferous tubule. The central luminal part itself was found to contain spermatids at various stages of spermatogenesis usually dislodged and placed free in the lumen. In certain sections of the seminiferous tubules, the spermatocytes and spermatids were seen to intermingled thus indicating complete disruption of the sertoli cells which otherwise keep the spermatocytes and spermatids in proper order of distribution in the seminiferous tubules (Fig. 1; 1B, 1E and 2B1).

In the Pb+M treated group partial restoration of the interstitial tissue, regeneration of the spermatogonia that obviously increased the number of these cells along the basement membrane were very much clear. The spermatocytes were found to be at various stages of rehabilitation in various sections that is almost complete normal arrangement of spermatocytes and spermatids one hand with enlarged vacuolated spermatocytes and number of spermatids in between two complete destructions forming a thick halo of cellular debris on the other extreme (Fig. 1; 1C and 1F).

In the Pb+J group seminiferous tubules appeared more ellipsoidal in outline with signs of restoration of spermatogenesis and indicated by the presence of spermatids and differentiated spermatids and differentiated spermatozoa towards lumen of tubules. The interstitial tissue although have shown more in number of cells as compared to lead group. With slightly smaller in size still show scattered distribution with focal empty spaces indicating a sort of mitotic rehabilitation of interstitial tissue shown in Fig. 2. (2C1). The miero-metric results of testes among the groups as shown in Table 1.

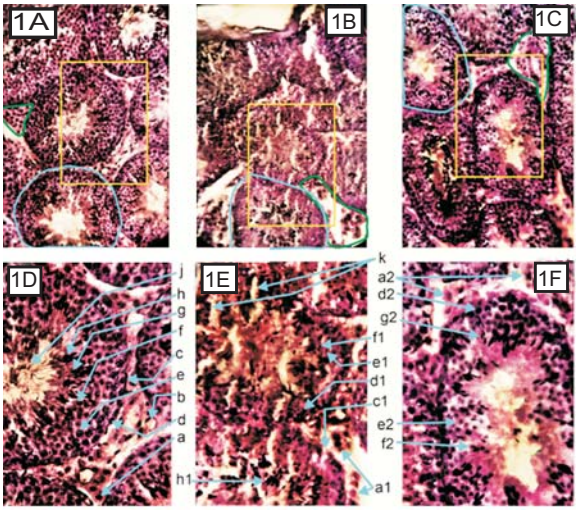

Fig. 1. Selected section of testes (400X)from,1A = control; 1B = Pb group; 1C = Pb+ *Morus* group: Green encircled area in slide 1A = Normal healthy interstitial tissue; In slide 1B = Interstitial cells necrosis and fluid accumulation and in slide 1C = Interstitial tissue regeneration.Blue encircled areas in slide 1A = CS of control seminiferous tubule showing normal spermatogenesis and intact basement membrane; 1B = CS of seminiferous tubule showing disrupted basement membrane and spermatogenesis; 1C = Partal rehabilitation of spermatogenesis. Yellow rectangular areas in 1A,1B and 1C = Cropped and digitally enlarged in sides 2A 2B; a = well developed interstitial tissue cells; a1 = degenerated interstitial tissue; a2 = partially repaired interstitial tissue; b = blood vessels; c = normal intact basement membrane, c1= disrupted basement membrane; d = spermatogonia; d1 = dislodged of spermatogonial cells; d2 = partial rehabilitation of the spermatogonial cells; e = primary spermatocyte; e1 = necroting primary spermatocytes; e2 = primary spermatocytes; f = secondary spermatocyte; f1 = secondary spermatocyte necrosis; f2 = reappearance of secondary spermatocytes (indicative of the rehabilitation of spermatogenesis); g = spermatids; g2 = newly appeared spermatids; h = differentiating spermatozoa (tail development); h1 = spermatozoa with no tail development; j = fully formed and dislodged spermatozoa present in the lumen of the seminiferous tubule; k = internal lesions of the seminiferous tubules with a propable necrosis of the nurse (sertoli) cells.

Lead is the environmental toxicant adversely affecting on male reproductive system, especially on testicular spermatogenesis and epididymal spermatozoa (Kumar, 2018). Traditionally lead has been reported to cause different toxicological actions in animal cells, tissues

and organ (Abdulrazzaq *et al*., 2016). In present study a significant decrease in body weight was found due to lead and there is no significant difference in mean testicular weight. It is reported that lead cause significant decrease in body weight of animals as compared to control (Bouazza *et al*., 2018).

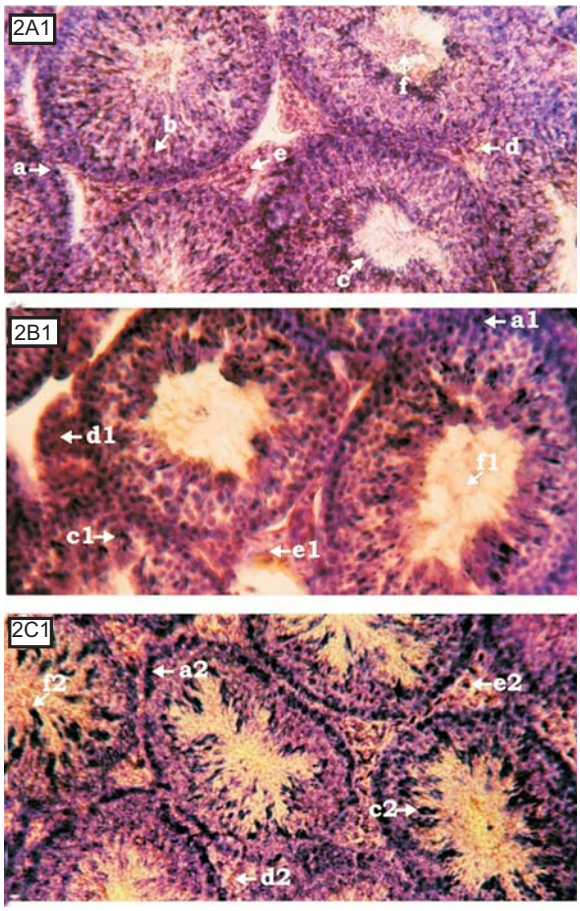

Fig. 2. Selected section of testes (400X) from, A1 = Control; B2 = Pb group; C1 = Pb+jambul group; a = healthy spermatogonia; a1 = dislodged spermatogonia; a2 = partial rehabilitation of the spermatogonial cells; b = spermatocyte; c = differentiating spermatozoa; c1 = dislodged spermatozoa; c2 = fully formed spermatozoa; d = well developed interstitial tissue; d1 = shrinked interstitial tissue; d2 = partially rehabilitative interstitial tissue;e = leydig's cells; e1 = lesser number of leydig's cells;e2 = regenerated leydig's cells; f = lumen of seminiferous tubules filled with spermatozoa; f1 = hollow lumen of seminiferous tubules devoid of spermatozoa; f2 = lumen of seminiferous tubules partially filled with developing spermatozoa.

In present study results have shown drastic pathological changes in testicular histology upon sub chronic lead exposure that include death of the interstitial tissue leaving wide gaps between the adjacent sections of seminiferous tubules. In most of such cases signs of cellular vacuolations and apoptosis were very obvious. These findings are well in line with the previous research reports such as lead could disturb mitosis *in vivo* in spermatogenic cells and alter sertoli cells proliferation, destruction of interstitial tissue and produce significant decrease the sperm functions in testis (Meroni *et al*., 2019). Higher concentration of lead interrupts the maturational changes of the spermatozoa, reduction in the spermatogonia and reduced number of Leydig cells (Chaithra *et al*., 2020).

The control group has rounded seminiferous tubules with proper distribution of spermatogonia, spermatocytes, spermatids and differentiated spermatozoa arranged from base to center of tubules. Among tubules healthy cells of interstitial tissues were found evenly distributed. In another study there was a healthy sign of seminiferous tubules development and even distribution of cells in control group (Heinrich and Defalco, 2020). Results have shown a significant decrease in average number of seminiferous tubules in Pb group. Another study also investigated that Pb induced superficial damage and decrease in the number of seminiferous tubules (Anjum *et al*., 2017). Pb treatment induced disorder in arrangement of spermatogenic cells due to which epithelial height was increased and lumen was decreased.

Jambul and Morus exposure has been seen to promote the halted process of spermatogenesis, in mice testis there by indicating its key role to eradicate the possible Pb induced disruption of microtubular polymerization and thus disruption of spermatogenesis, possibly through Pb ion chelation by its polyphenolic ingredients (Mêzyñska and Brzóska, 2018).The recovery of these pathological signs upon post treatment of MFE and JFE in terms of the partial rehabilitation of the interstitial tissue, regeneration of the spermatogonia that obviously increased the number of these cells along the basement membrane indicates the medicinal importance of this

Table 1. Shows different micrometric results of testes among the groups (values are mean±SEM, n=10)

Micrometric parameters	Control	Lead	Lead+ *Morus alba*	Lead+ *Syzygium cumini*
Mean body weight	33.80±0.60[b]	28.94±0.56[a]	32.36 ±0.62[b]	31.30±0.59[b]
Mean organ weight	0.26	0.18	0.25	0.23
Average CSA of seminiferous tubule	11693.7[b]	17268.7[a]	12208.2[b]	11636.91[b]
Number of seminiferous tubule (per 10,000,000μ^2)	14.5[b]	7.6[a]	10.08[ab]	11.86[b]
Seminiferous epithelial height	23.22[b]	45.94[a]	25.12[ab]	23.11[b]
Number of spermatogonia (per 1,183,744μ^2)	60.8± 0.5[b]	43.00± 0.5[a]	53.58±0.5[ab]	56.0±0.9[c]
No. of spermatogonia***	10±2.02[c]	2.1±1.9[a]	4.8±1.4[b]	4.9±1.2[b]
No. of primary spermatocytes***	9.3±1.5[c]	1±1.1[a]	5.9±2.5[b]	6.2±2.3[b]

Comparing the same histometric parameter, the vehicle control (C), Lead (Pb), Lead+*Syzygium cumini* (Pb+J): Lead+*Morus alba* (Pb+M): *P<0.001; comparing, for the same histometric parameter, any two groups not sharing a common lower-case superscript, a, b, c: P<0.05.

fruit in testicular pathologies. The previous reports also support these findings. In a study it was reported that *Morus alba* showed normal process of spermatogenesis with normal arrangement of spermatocytes (Dkhil *et al*., 2015). Evidence indicates that *Morus alba* can exert several health beneficial effects such as antibacterial, hepatoprotective, immunomodulatory, anti-inflammatory and reproductive effects. The antioxidant potency of some phenolic compounds from *Morus alba* has been reported in different experimental models (Rodrigues *et al*., 2019).

Conclusion

Based upon these findings it is concluded that Pb possess organ toxic capacities causing characteristic alterations in testicular histology that can be rapidly rescued through MFE treatment indicating the importance of *Morus alba* fruit in male reproductive health.

Conflict of Interest. The authors declare that they have no conflict of interest.

Refrences

Abdulrazzaq, A.M., Hezmee, M.N.M., Abd Wahid, H.A.W., Sabri, M.Y.M., Rajion, M.A. 2016. The detrimental effects of lead on human and animal health. *Veterinary World*, **9:** 660-671.

Anjum, M.R., Madhu, P., Reddy, K.P., Reddy, P.S. 2017. The protective effects of zinc in lead induced testicular and epididymal toxicity in wistar rats. *Toxicology and Industrial Health*, **33:** 265-276.

Bouazza, S., Demmouche, A., Toumi-Benali, F., Zouba, M., Rabie, B.M., Agher, N., Merakchi, N., El Ahmar, M. 2018. Effect of bee pollen extract on lead induced toxicity in rat testis. *South Asian Journal of Experimental Biology*, **8:** 91-102.

Chaithra, B., Sarjan, H.N., Shivabasavaiah. 2020. A comparative analysis of fluoride contaminated groundwater and sodium fluoride induced reproductive toxicity and its reversibility in male rats. *Biological Trace Element Research*, **197:** 507-521.

Chhikara, N., Kaur, R., Jaglan, S., Sharma, P., Gat, Y., Panghal, A. 2018. Bioactive compounds and pharmacological and food applications of *Syzygium cumini*–a review. *Food Function*, **9:** 6096-6115.

Dkhil, M., Bauomy, A.A., Diab, M.S.M., Al-Quraishy, S. 2015. The antioxidant effect of *Morus alba* leaves extract on kidney, testes, spleen and intestine of mice. *Pakistan Journal of Zoology*, **47:** 393-397.

Gomes, M., Goncalves, A., Rocha, E., Sa, R., Alves, A., Silva, J., Barros, A., Pereira, M.L., Sousa, M. 2015. Effect of *in vitro* exposure to lead chloride on semen quality and sperm DNA fragmentation. *Zygote*, **23:** 384-93.

Heinrich, A., Defalco, T. 2020. Essential roles of interstitial cells in testicular development and function. *Andrology*, **8:** 903-914.

Hu, Y., Zhou, J., Du, B., Liu, H., Zhang, W., Jiani, L., Zhang, W., You, L., Zhou, J. 2019. Health risks to local residents from the exposure of heavy metals around the largest copper smelter in China. *Ecotoxicology Environmental Safty*, **171:** 329-336.

Kianoush, S., Sadeghi, M., Balali-Mood, M. 2015. Recent advances in the clinical management of lead poisoning. *Acta Medica Iranica*, **53:** 327-36.

Kumar, S. 2018. Occupational and environmental exposure to lead and reproductive health impairment: *Indian Journal of Occupational and Environmental Medicines*, **22:** 128-137.

Lanjewar, A.M., Sharma, D.V.K., kosankar, P, K., Thomber. 2018. Extraction and phytochemical Screening of *syzygium cumini* seeds in Vidarbha region of india. *World Journal Pharmacetical Research*, **7:** 1782-1791.

Latif, W.A., Ara, A., Usmani, J.A. 2015. Lead toxicity: a review. *Interdisciplinary Toxicology*, **8:** 55-64.

Li, C., Zhao, K., Zhang, H., Liu, L., Xiong, F., Wang, K., Chen, B. 2018. Lead exposure reduces sperm quality and DNA integrity in mice. *Environmental Toxicology*, **33:** 594-602.

Meroni, S.B., Galardo, M.N., Rindone, G., Gorga, A., Riera, M.F., Cigorraga, S.B. 2019. Molecular mechanisms and signaling pathways involved in sertoli cell proliferation. *Endocrinology*, **10:** 224 https://doi. org/10.3389/fendo.2019.00224.

Mêzyñska, M., Brzóska, M.M. 2018. Review of polyphenol rich products as potential protective and therapeutic factors against cadmium hepatotoxicity. *Applied Toxicology*, **39:** 117-145.

Rodrigues, E.L., Marcelino, G., Silva, G.T., Figueiredo, P.S., Garcez, W.S., Corsino, J., Rita de Cássia Avellaneda Guimarães., Karine de Cássia Freitas. 2019. Nutraceutical and medicinal potential of the *Morus* Species in metabolic dysfunctions. *International Journal of Molecular Sciences*, **20:** 301.

Santhosh, K.R., Asha, D.S. 2018. Lead toxicity on male reproductive system and its mechanism: a review. *Research Journal of Pharmacy and Technoloy*, **1:** 1228- 1232.

Singh, B., Singh, J.P., Kaur, A., Singh, N. 2018. Insights into the phenolic compounds present in jambolan (*Syzygium cumini*) along with their health promoting effects. *International Journal of Food Science and Technology*, **53:** 2431-2447.

Takeshima, T., Usui, K., Mori, K., Asai, T., Yasuda, K., Kuroda, S., Yumura, Y. 2021. Oxidative stress and male infertility. *Reproductive Medicine and Biology*, **20:** 41-52.

Ustundag, A.O., Ozdogan, M. 2016. Usage possibilities of mulberry leaves in poultry nutrition. scientific papers. *Series D. Animal Sciience*, *VIII*. ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750.

Yuan, O., Zhao, L. 2017. The Mulberry (*Morus alba* L.) Fruit-a review of characteristic components and health benefits. *Journal of Agriculture of Food Chemistry*, **65:** 10383-10394.