Pak. j. sci. ind. res. Ser. B: biol. sci. 2022 **65B**(3) 222-227

Study of Histo-curative Potential of Jambul (*Syzygium cumini*) Fruit Pulp Extract in Mice Kidney Under Chromic Lead (Pb) Exposure

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(received October 22, 2020; revised March 13, 2021; accepted April 1, 2021)

Abstract. Lead oral exposure related pathological changes in kidneys and their ameliorations through Jambul Fruit Pulp Extract (JFPE) treatment were investigated in Albino Laboratory Mice. Adult male animals (n=30) weighing an average of 30 g were equally distributed into three groups, control (no treatment), lead (Pb) group (50 ppm Pb ions from lead acetate were given in drinking water 15 days+Pb ions free water 5 days) and Pb-J (lead+Jambul) group (50 ppm Pb ions as in Pb group+Jambul pulp extract 0.2 mL from 16-20 days). All animals were euthanized to recover kidneys for histopathological study on day 21. Results showed Pb exposure related significant decline in kidney and animal body weights. Histopathological findings were shrinkage of glomeruli and the proximal tubules, whereas the micrometric data indicated significant decline in glomerular size (2233.91±47.9 μm^2) CSA (cross sectional area) of tubule size (561.0±23.4 μm^2), caliber (97.9±7.2 μ^2), wall thickness (462.0±22.8 μ^2) and number of glomeruli per unit area (1.56±0.2) in Pb group. All these weight parameters, histopathological signs and micrometric observations indicate very good signs of recovery from such pathological changes in Pb-J group. These findings clearly show the importance of JFPE towards rehabilitation of the functional micro-architecture of the kidneys after Pb ion exposure related damage.

Keywords: histo-curative, *Syzygium cumini*, kidney, renal histopathology, lead

Introduction

Lead (Pb) is a heavy metal with soft consistency and gives greyish blue tint. It causes a wide range of dysfunctions both physiological as well as biochemical (Kanupuru and Kumari, 2016) and accumulates in various tissues (Shahsavani *et al*., 2011). It pollutes through industrial discharge, automobile fuels and paints (Aucott and Caldarelli, 2012). Lipid per-oxidation is the mechanism of cellular injury which increases oxidative stress in cells and tissues. It degrades the polysaturated fatty acids of cell membrane resulting in their disruption (Zhang *et al*., 2015). In blood, 95% of lead (Pb) is bound to erythrocytes and rest stays in plasma (Radulescu and Lundgren, 2019).

Kidneys are target sites for lead toxicity (Rastogi, 2008) especially the cortex (Viñas *et al*., 2018). They are vulnerable to toxic injury, directly from exposure through open fenestrae of glomerular capillaries (Xu *et al*., 2018). Lead nephrotoxicity is characterized by proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis (Peres *et al*., 2019).

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Many plant products have antioxidant activities such as free radical scavenging, inhibiting lipid per-oxidation (Wang *et al*., 2020) and need to be studied. Jambul (*Syzygium cumini*) belongs to family Myrtaceae and is a perennial flowering plant (Balyan and Sarkar, 2017). Jambul has deep purple colour due to anthocyanins. It contains vitamin C, gallic acid, tannin and anthocyanins including cyanidin, petunidin and malvidin glucoside (Gajera *et al*., 2018; Liya *et al*., 2009). Anthocyanins inhibit lipid per-oxidation and platelet aggregation (Sharifiyan *et al*., 2016). Liver and kidneys are best animal-derived source of vitamin C (Lee *et al*., 2016). Ameliorative properties of Jambul fruits on lead affected kidney cells are known and this study was carried out to gather for further evidence in this domain.

Materials and Method

Animal keeping. Male laboratory mice of albino variety (*Mus musculus)* were utilized for current research and kept under equal dark/light shifts of 12 h. Room temperature was maintained (23 ± 2 °C). Feed included dried milk, water and required vitamins.

Dose groups. 30 male mice having similar weights (30 g) were further divided into three groups (each containing 10 animals).

Control (untreated) group (Co). This group was provided regular feed and drinking water throughout the study period (20 days).

Lead treated group (Pb). These animals were given 50 ppm of Pb-ions in drinking water for 15 days and Pb free water for next 5 days.

Pb and Jambul extract co-treated group (Pb-J). These animals were given Pb ions (50 ppm) in drinking water for 15 days and 0.2 mL JFPE regularly at 12 h intervals from day 16-20 along with Pb free water.

Dose preparation. Lead acetate ($PbCH_3COO$) 1.56 g was dissolved in 1 liter water to achieve 1000 ppm Pb ions stock solution. By adding 950 mL of water to 50 mL stock solution, required dose (50 ppm) was prepared. 100 g of JFPE (Jambul Fruit Pulp Extract) was blended with 100 mL. One g/mL of drinking mineral water for 10 min to obtain juice. Fibrous content of juice was removed through centrifuge and supernatant frozen at 30 °C for consumption of Pb-J group animals (Inayat *et al*., 2020).

Organ recovery. Kidneys were removed on 21st day of experiment by dissection. Kidneys obtained were weighed and finally fixed in Cornoy's fixative for further study.

Histological preparations and observations. Wax embedding of collected kidneys was carried out. Transverse sections of (5 µ thick) blocks were obtained and stretched on albumenized histological glass slides. E and H staining was done and canada balsam mounting carried out. Slides were observed for Pb related histopathology and effects of fruit extract against Pb induced alterations.

Digital photography and processing. Photomicrographs were obtained using digital Sony camera. 100× and 400× magnifications were used and subsequent processing carried out through Corel DRAW 11. Selected histological sections of kidney belonging to different groups (Co, Pb¸ Pb-J) were thus prepared (Ahmad *et al*., 2016).

Micrometry. Digital micrometry was carried out with soft images (400x) of 30 randomly selected sections of kidneys from each group. Measurements of CSA of glomeruli etc. were taken from each section using digital scales and micrometric data obtained was used to calculate group means ± SEM values. Thus measurement were made for Average Cross Sectional Area (ACSA) of glomeruli, ACSA of tubules, ACSA of tubular caliber, ACSA of tubular wall and number of glomeruli per unit (159737.707 μ^2) area. The ACSAs were calculated using following formula:

$$ACSA = (\text{length} \times \text{width}/4\pi)$$

Data analysis. Statistical analysis was based on single factor ANOVA and further on Duncan's Multiple Range Test for group comparison. Histograms were utilized for data presentation.

Results and Discussion

Body weight. Mean body weight of animals belonging to Co, Pb and Pb-J treated groups are given in Table 1. At the start of experiment, mean initial body weight of all experimental group animals showed slight variation. At the end of experiment, Pb treatment showed highly significant ($P \leq 0.001$) decrease in mean body weight of animals as compared to Co while in Pb-J treated group, no significant reduction was observed compared to Co. Within groups, Pb treatment resulted in highly significant ($P \leq 0.001$) decrease in mean final body weight compared to Pb-J treatment. Data analysis revealed toxic effect of lead on body weight of animals at the given dose level and this toxic effect was observed to be decreased by the post treatment of JFPE which showed the protective ability of jambul fruit against lead like toxicants.

Organ weight. Mean kidney weight of Co. Pb and Pb-J treated groups are mentional in Table 2. One way analysis of variance showed that Pb treatment resulted in significant ($P \leq 0.05$) decrease in mean kidney weight compared to Co group. There was slight non-significant reduction in lead post treated with Jambul reflecting

Table 1. Mean body weight of animals belonging to groups Co, Pb and Pb-J treated groups

	Animal body weight (g)	
Groups	Initial weight	Final weight
Co	29.58±0.51	32.98±0.71^a
Pb	30.42±0.38	27.12±0.45^b
Pb-J	29.86±0.26	31.22±0.39^a

Values are expressed in mean ± SEM. Any two columns in the group not sharing a common superscript alphabet vary significantly. As indicated by means of Duncan's Multiple Range Test ($P \leq 0.05$).

protective role of fruit pulp extract against Pb toxicity. Among groups, significant reduction in mean kidney weight in Pb treated group was noticed as compared to Pb-J treated group.

Histological observations. The micro-anatomical layout of histological sections of kidney in control group were showing all landmarks of normal structural distribution such as rounded glomeruli with appropriate peri-glomerular spaces, embedded in the rounded sections of proximal nephronal convolutions. The nephronal tubules appear healthy with central calibers inside (Fig. 1a). On the other hand, kidney sections in lead treated group have shown various pathological changes such as shrinking or atrophied glomeruli showing widened peri-glomerular spaces. The proximal tubules show swelling with almost completely obliterated caliber at various places (Fig. 1b). Tubular necrosis was also observed in Pb-J treated group. Many signs of recovery were observed which include rehabilitation of glomeruli structure, restoration of the proximal tubular disposition and infiltrations of large number of stem cells with characteristic rounded and less densely stain nuclei indicating a sort of regenerative process (Fig. 1c).

Table 2. Mean kidney weight of Co, Pb and Pb-J treated groups

Organ weight (g)	
Groups	Weight
Co	0.37±0.02[a]
Pb	0.26±0.01[b]
Pb-J	0.33±0.01[a]

Values are expressed in Mean ± SEM. Any two columns in the group not sharing a common upper case alphabet vary significantly. As indicated by means of Duncan's Multiple Range Test ($P \leq 0.05$).

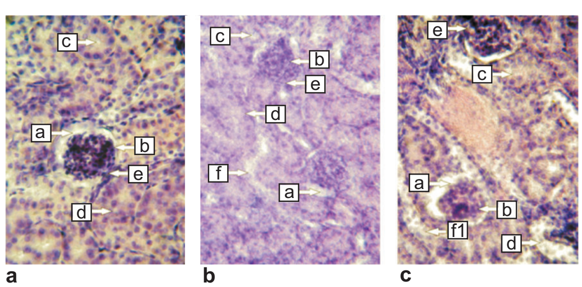

Fig. 1. Selected sections of kidneys from **(a)** control, **(b)** Pb, **(c)** Pb-J groups; a = urinary space in Bowman capsule; b = normal glomeruli; c = proximal tubule; d = distal tubule; e = vascular pole; f = damaged tubule; f_1 = repairing tubule.

Micrometric results of kidney. ***CSA of glomeruli.*** Highest mean CSA of glomeruli was recorded in control (2571.5±63.9 μ^2), followed by Pb-J treated group (2336.09 μ^2) and least in Pb treated group (2233.91 μ^2). Statistical analysis (ANOVA) has shown significant variation among the groups ($P \leq 0.0001$). The post hoc analysis indicated no significant ($P \leq 0.05$) difference between Pb and Pb+JFPE, while they differ significantly with Co group (Fig. 2).

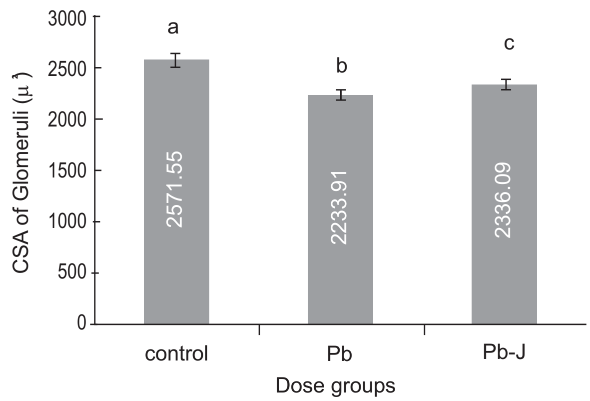

Fig. 2. Mean CSA of glomeruli of control, Pb and Pb-J treated groups. Plus bar indicate mean±SEM. Any two columns among the groups not sharing a common lowercase letter have shown significant variation. As indicated by means of Duncan's Multiple Range Test ($P \leq 0.05$).

CSA of tubular area. Highest mean CSA value of tubular area was recorded in control (863.0±24.9 μ^2), followed by Pb-J treated group (729.2±22.1 μ^2) and least CSA of tubular area was noticed in Pb treated group (561.0±23.4 μ^2). Statistical analysis (ANOVA) has shown highly significant variation among the groups ($P \leq 0.0001$). The post hoc analysis indicate significant ($P \leq 0.05$) difference among all these groups (Fig. 3).

CSA of tubular caliber. Highest mean CSA value of tubular caliber was observed in control (199.3±7.2 μ^2), followed by Pb-J treated group (176.8±4.1 μ^2) and least CSA of tubular caliber was noticed in Pb treated group (97.9±7.2 μ^2). Statistical analysis (ANOVA) has shown highly significant variation among the groups

(P≤0.0001). The post hoc analysis shows significant (P≤0.05) difference with each other (Fig. 4).

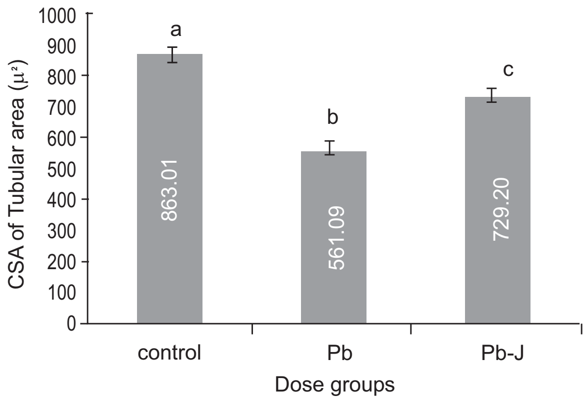

Fig. 3. Mean CSA of tubular area in control, Pb and Pb-J treated groups. Plus bar indicate mean±SEM. Any two columns among the groups not sharing a common lowercase letter have shown significant variation. As indicated by means of Duncan's Multiple Range Test (P ≤ 0.05).

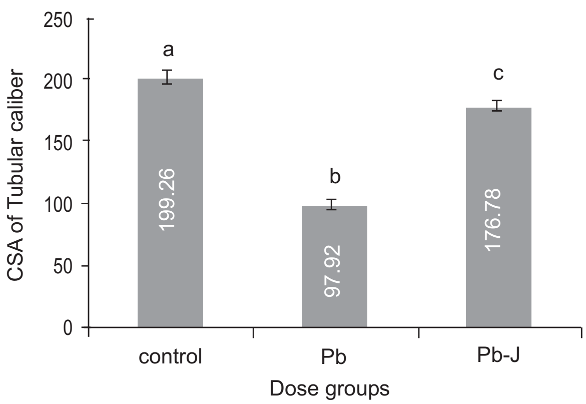

Fig. 4. Mean CSA of tubular caliber in control, Pb and Pb-J treated groups. Plus bar indicate mean±SEM. Any two columns among the groups not sharing a common lowercase letter have shown significant variation. As indicated by means of Duncan's Multiple Range Test (P ≤ 0.05).

CSA of wall area. Highest mean CSA value of wall area was observed in control (657.9±21.3 μ^2), followed by Pb-J treated group (555.5±20.6 μ^2) and least CSA of wall area was noticed in Pb treated group (462.0±22.8 μ^2). Statistical analysis (ANOVA) has shown highly significant variation among the groups (P≤0.0001). The post hoc analysis shows significant (P≤0.05) difference with each other (Fig. 5).

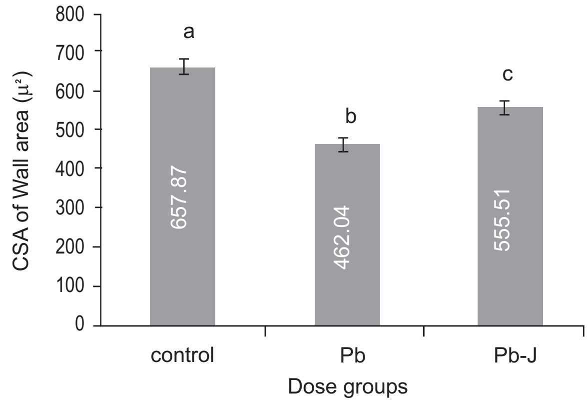

Fig. 5. Mean CSA of wall area in control, Pb and Pb-J treated groups. Plus bar indicate mean±SEM. Any two columns among the groups not sharing a common lowercase letter have shown significant variation. As indicated by means of Duncan's Multiple Range Test (P ≤ 0.05).

Number of Glomeruli per unit (159737.707 μ^2) Area. Highest number of glomeruli was observed in Pb-JFPE (2.2±0.1), followed by control (2.0±0.6) and least number of glomeruli were noticed in Pb treated group (1.56±0.2). Statistical analysis (ANOVA) has shown highly significant variation among the groups (P≤0.01). The post hoc analysis shows significant (P≤0.05) difference among Pb and Pb-J treated groups (Fig. 6).

Lead is one of the most common heavy metal having the risk of environmental exposures. It has long been reported to induce various toxicological changes in experimental animals. It has been found to be absorbed readily through GI tract on oral exposure and accumulates in soft tissues like liver, kidney, brain and spleen (Abedi *et al*., 2020; Durgut *et al*., 2008).

In present study significant decline in animal body weight was noted with lead exposure. It has long been postulated that result and body weight loss in lead group animals is a consequence of nausea, anorexia and vomiting induced by lead exposure (Amjad *et al*., 2013). Characteristic damage to the kidney is significant loss in kidney weight, glomerular atrophy, shrinkage of

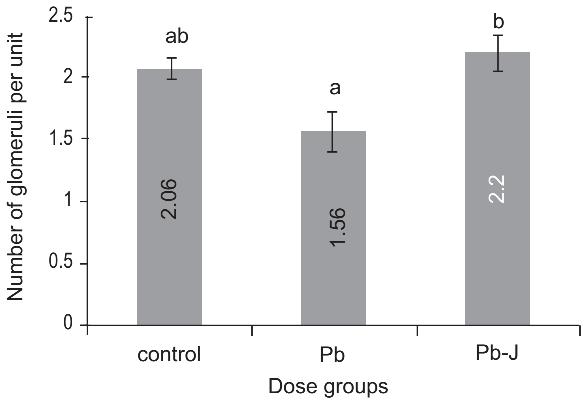

Fig. 6. Number of glomeruli per unit area in control, Pb and Pb-J treated groups. Plus bar indicate mean±SEM. Any two columns among the group not sharing a common lowercase letter have shown significant variation. As indicated by means of Duncan's Multiple Range Test ($P \leq 0.05$).

kidney tubules and significant reduction in functional capacity of kidney tubules as reflected by the number of glomeruli per unit area.

Jambul fruit pulp extract has already been found beneficial against various toxicological environmental substances (Gordon *et al*., 2011). One possible way of rescue against these implications is the presence of variety of antioxidants (Anthocyanins, Vitamin C, Flavonoid and Phenolic acid) in it that reduce the chances of toxicological process mediated through oxidative stress (Lo *et al*., 2010; Talas *et al*., 2009). In the present study, post treatment of Jambul has been found to rescue from general body weight and kidney weight loss. Histological results show rehabilitation of glomeruli structure, restoration of the proximal tubules and infiltration of large number of stem cells. The micrometric signs show increased CSA of glomeruli, CSA of tubular area, CSA of tubular caliber, CSA of wall area and number of glomeruli per unit area.

Conclusion

On the basis of results obtained it is concluded that lead (Pb) exposure at a dose level of 50 ppm or more in drinking water may lead to various types of toxicological changes in histo-architecture of the kidney. However all toxicological changes were recovered on Jambul pulp extract post treatment indicating the medicinal importance of JFPE (Jambul fruit pulp extract) against toxicological exposure of heavy metals such as lead.

Acknowledgment

Authors are thankful to the authorities of University of Sargodha for providing animal house and laboratory facilities.

Conflict of Interest. The authors declare no conflict of interest.

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