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Rescuing Potential of *Syzygium cumini* and *Morus nigra* Fruit Extracts On Hexavalent Cr Induced Anomalies of Kidney in Mice

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Abstract. The present histopathological study was conducted to reveal the ameliorative potential of *Syzygium cuminin* and *Morus nigra* fruit extracts in response to hexavalent chromium Cr (VI) induced renal toxicity. Mice were divided into four study groups *i.e*., control (Ctl) group, chromium (Cr) treated group that was given 50 ppm chromium given *ad labitum* for 10 days, chromiun+Jambul group (Cr+Jb) given 50 ppm (Cr) for 10 days followed by 0.2 mL/12 h of fresh Jambul fruit extract for 5 days and chromium+*Morus* (Cr+Mr) group given 50ppm (Cr) for 10 days followed by 0.2 mL/12 h of fresh *Morus* fruit extract for next 5 days. For renal histopathological study kidney was recovered on 15th day of study. In Cr (VI) group, the glomeruli are rounded and highly stained along with t atrophy and necrosis in Cr group including tissue damages *i.e*. shrinkage along with peripheral fibrosis of the renal glomeruli and fairly large number of glomerular obliterations. In Cr+Jb and Cr+Mr group, rapid reversal of the nephropathological signs. The inter-tubular spaces were reduced and renal tubules regained the size in Cr+Jb and Cr+Mr group just like Ctl. These findings suggest that Cr is potentially toxic for animal kidney. Highest mean value for CSA of cortical glomeruli was found in Cr+Jb (3132.5± 266.9μ²) and CSA of medullary glomeruli was (3129.1±145.2μ²) as compared to cortical Ctl glomeruli (2441.7± 95.7μ²) & cortical Ctl glomeruli (2515.1.7±115.1μ²). However, such pathologies can rapidly be repaired upon *Syzygium cumini* and *Morus nigra*. pulp extract treatment. Thus it is clearly indicated that *Morus nigra* fruit extract possesses considerable excellent rescuing capacity against renal pathology of environmental toxins specifically Cr.

Keywords: *Syzygium cumini*, *Morus nigra*, anomalies, fruit extracts, kidney, Cr (VI)

Introduction

Cr (VI) is considered the most toxic form of chromium as it has high oxidizing potential, high solubility and mobility across the membranes (Yamagishi *et al*., 2017). It is involved in the redox cycle that produces reactive oxygen species (Handa *et al*., 2017). Owing to the production of reactive oxygen species and it is recognized as genotoxic, cytotoxic and carcinogenic (Chen *et al*., 2019). The nephrotoxic effects of the heavy metal chromium are well elaborated in the literature (Tsai *et al*., 2017). Chromium is reported to induce renal necrosis along with abnormal mitochondrial dynamics in mice (Zheng *et al*., 2020).

Jambul (*Syzygium cumini* from Myrtaceae family) is blackish when ripen out due to the presence of the pigment anthocyanin which is an antioxidant (Ahmad *et al*., 2017). Jambul contains compounds like myricetin (a flavonol), bergenin (an isocoumarin), alkaloids (Jambosine), tannins, triterpenoids and volatile oils (Chhikara *et al*., 2018). Mulberry fruits of the family Moraceae are widely used as nutritious food and rich sources of antioxidant compounds having beneficial effects on health. Its fruit has excellent quality and high nutritive value, widely used in the human diet, medicine and industrial processing (Rodrigues *et al*., 2019). Jambul is shown to possess excellent healing effects on kidneys (Ahmed *et al*., 2017). *Morus* is also recognized as an ameliorative agent against nephrotoxicity (Hassanalilou *et al*., 2017). This study is conducted to investigate the potential histopathological damages of Cr on kidney health and possible rescuing potential of natural antioxidants present in *Syzygium cumini* and *Morus nigra*.

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Table 1. Important phytochemical ingredients of fruit *Syzygium cumini* and *Morus nigra*

Fruit	Phytochemicals	Biologically active phytochemicals of Jambul and *Morus* fruit
Jambul (*Syzygium cumini*)	**Acid:** malic acid, oxalic acid (Dagadkhair *et al*., 2017): Galic acid, tannins: **Sugar:** glucose,fructose, mannose, **glactose:** Mineral: Mg, Na, Ca, Cu, K, Fe: Vitamin: vit. A, riboflavin, thiamine, nicotinic acid, vit. C, folic acid, nicotinic acid: **Anthocyanin:** delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside, cyanidindiglycoside, petunidin, malvidin: **Flavanoids:** Myricetin, myricetindeoxyhexoside (Hassan, 2018)	Gallic acid, malic acid, malvidin,myricetin,myricetindeoxyhexoside,delphinidin-3-gentiobioside (Hassan, 2018)
Black mulberry (*Morus nigra*)	**Fatty acid:** linoleic acid, palmitic acid, oleic acid: **Sugar:** glucose, fructose: **Anthocyanin:** cyanidine-3-glucoside, cyanidine-3-rutinoside (Wen *et al*., 2019: Espada-Bellido *et al*., 2017) **Vitamin:** Vit. K, Vit. C, Niacin, Riboflavin, **Pyridoxine:** Mineral: Potassium, Iron, magnesium: Flavanoid: Morin, **Phenol:** resveratrol (Memete *et al*., 2022)	Cyanidine-3-glucoside, cyanidine-3-rutinoside, resveratrol (Wen *et al*., 2019)

Materials and Methods

Animal rearing, maintenance and feeding. Male albino labouratory mice of average 30g weight reared in the animal house (with controlled temperature, humidity and day and light cycle) of University of Sargodha, Sargodha were used for the study.

Dose preparation. Stock solution of 1000ppm (2.82g of $K_2Cr_2O_7$ in 1000mL water) was prepared and then diluted further to prepare 50ppm solution.

Fruit extract dose. Fresh *Syzygium cumini* and *Morus nigra* were obtained from local market and their juice was made. It was then centrifuges to obtain supernatant. It was stored at -20 °C.

Dose groups. Animals were divided into four groups (each with 10 animals of 30g weight).

- **Control (Ctl) group.** animals of this group were given plain drinking water for 15 days.
- **Chromium (Cr) group.** This group was given 50ppm Cr dissolved in drinking water for 10 days followed by plain water for rest of 5 days.
- **Chromium and Jambul (Cr+Jb) group.** 50ppm Cr was given to this group for first 10 days, followed *Syzygium cumini* fruit extract (0.2mL/12h) by gavage for next 5 days.
- **Chromium and Morus group (Cr+Mr).** 50ppm Cr was given for first 10 days, followed by *Morus nigra* fruit extract (0.2mL/12 h) by gavage for next 5 days.

Histological preparations and observations. Kidney was processed of for wax embedding and serial transverse sections of (5 microns) were obtained on a rotary microtome (Erma Tokyo 422). These serial transverse sections were stained using Eosin hematoxylin staining. These slides were finally observed under trinocular microscope at 400x magnification.

Digital photography and micrometry. Digital photographs of the selected slides of different groups (Ctl, Cr, Cr+Jb and Cr+Mr) were taken using a digital Sony camera of 7.2 mega pixel fitted on a Labomid CXR2 trinocular microscope. A computer-based technique using CorelDraw11 was used to generate a micrometric data from the digital photomicrographs of the histological sections of kidneys.

Analysis of data. Digital histometry of the selected histological sections of kidney were conducted in CorelDRAW11® under calibrated scales in microns (µ). Data obtained through histometry was analyzed by IBM SPSS Statistics 23 using ANOVA (2-way) and Tukey's Multiple Range Test.

Results and Discussion

Histological observations. Histological slides at 400x after HE-Staining indicate normal architecture of glomeruli and other portion of renal tubules. The glomeruli are rounded with least inter-tubular space (Fig 1 A-a,b,c). Cr group indicated prominent atrophy and necrosis in glomeruli (Fig 1.B-d). The pathological signs observed in Cr group sections include shrinkage along with peripheral fibrosis of the renal glomeruli, fairly large number of glomerular obliterations was also observed. The dilated and vacuolated cells are prominent (Fig 1.B-d). There is inter-tubular space and shrinkage of renal proximal and distil tubules (Fig. 1.C-c). In Cr+Jb and Cr+Mr Group (Fig. 1.C- D-c) the glomeruli are rounded and highly stained. The inter-tubular spaces were reduced and renal tubules regained the size like control.

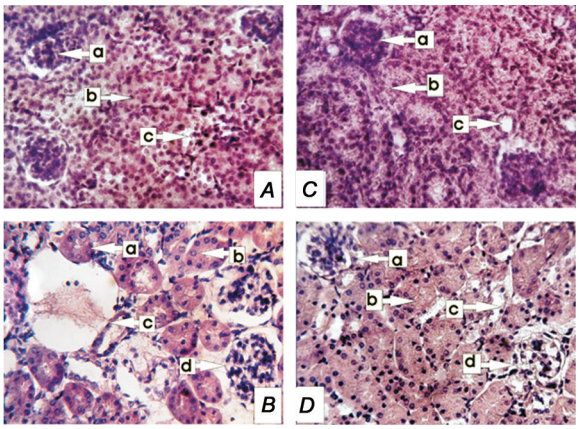

Fig. 1. Histological slides of mice kidney at 400x, A= control group (Ctl); B= chromium treated group (Cr); C= chromium post treated Jambul group (Cr+Jb); D= chromium post treated *Morus* group (Cr+Mr) [a= normal glomerulus; b= renal tubules, c: inter-tubular space; d= apoptotic glomerulus].

Micrometric observations. ***Number of glomeruli/ unit area in cortex.*** Highest mean value for number of glomeruli in cortex per 295936μ² was found in Ctl (2.0± 0.1) and Cr+Mr (2.2±0.1) followed by Cr+Jb (1.9±0.1) and Cr (1.7± 0.1).The statistical analysis showed no significant variation (P<0.102) among the groups.

Number of glomeruli/ unit area in medulla. Highest mean value number of glomeruli in medulla per 295936 μ^2 was found in Cr+Mr (2.3±0.1), Ctl (0.2±0.02) followed by Cr+Jb (0.2±0.01) and Cr (0.1±0.03).The statistical analysis showed a highly significant variation (P<0.000) among the groups. The post hoc analysis indicated a significant variation among Ctl and Cr+Jb; similarly between Cr and Cr+Jb, while there was no significant difference between Ctl and Cr.

CSA of cortical glomeruli. Highest mean value for CSA of cortical glomeruli was found in Cr+Jb (3132.5± 266.9μ²) followed by Ctl (2441.7± 95.7μ²) and Cr+Mr (2177.6±95.1μ²), while lowest for Cr (1916.07±92.9μ²) group. The statistical analysis showed a highly significant variation (P<0.000) among the groups. The post of hoc analysis indicated a significant variation among Ctl and Cr+Jb similarly between Cr and Cr+Jb and between Cr and Cr+Mr.

CSA of medullary glomeruli. Highest mean value for CSA of medullary glomeruli was found in Cr+Jb (3129.1±145.2μ²) followed by Ctl (2515.1.7±115.1μ²), Cr+Mr (2066.8±52.7μ²) and Cr (2169.1±128.9μ²) and the statistical analysis showed a highly significant variation (P<0.000) among the groups. The post hoc

Table 2. Micrometric readings of histological slides of study groups

Micrometric parameters	Groups			
	Ctl	Cr	Cr+Jb	Cr+Mr
CSA of Distal collecting tubules (DCT's) (μm^2)	787.3 ± 32.5^{b}	556.1 ± 25.1^{a}	733.9 ± 28.5^{b}	686.85 ± 41.68^{a}
CSA of Proximal collecting tubules (PCT's) (μm^2)	879.7 ± 46.6^{ab}	661.3 ± 39.4^{a}	1095.6 ± 91.9^{b}	661.7 ± 13.7^{a}
Number of glomeruli in cortex(295936 μ^2)	2.0 ± 0.1^{a}	1.7 ± 0.1^{a}	1.9 ± 0.1^{a}	2.2 ± 0.1^{b}
Number of glomeruli in medulla(295936 μ^2)	0.2 ± 0.02^{a}	0.1 ± 0.03^{a}	0.2 ± 0.01^{b}	2.3 ± 0.1^{a}
CSA of cortical glomeruli (μm^2)	2441.7 ± 95.7^{a}	1916.07 ± 92.9^{a}	3132.5 ± 266.9^{b}	2177.6 ± 95.1^{b}
CSA of medullary glomeruli (μm^2)	$2515.1.7\pm115.1^{a}$	2169.1 ± 128.9^{a}	3129.1 ± 145.2^{b}	2066.8 ± 52.7^{a}

The micrometric observations of the four study groups where mean value±standard error is shown. The alphabetical descriptions shows the results of posthoc tukey analysis.

analysis indicated a significant variation among Ctl and Cr+Jb similarly between Cr and Cr+Jb and Ctl and Cr+Jb.

CSA of proximal collecting tubules (PCT's). Highest mean value for CSA of PCT's was found in Cr+Jb (1095.6± 91.9μ²) followed by Ctl (879.7± 46.6μ²), Cr+Mr (661.7±13.7μ²) and Cr (661.3±39.4μ²).The statistical analysis showed a highly significant variation (P<0.000) among the groups.

CSA of distal collecting tubules (DCT's). Highest mean value for CSA of DCT's was found in Ctl (787.3± 32.5μ²) followed by Cr+Jb (733.9±28.5μ²) and Cr (556.1± 25.1μ²).The statistical analysis showed a highly significant variation (P<0.000) among the groups. The post hoc analysis indicated a significant variation among Ctl and Cr similarly between Cr and Cr+Jb, while there was no significant difference among Cr and Cr+Mr.

The Cr has been repeatedly reported to be nephrotoxic (Goodrazi *et al*., 2017). The tubular damage, particularly of the brush border in proximal tubules, individual cell necrosis in glomeruli and other structural changes have been reported (Venter *et al*., 2017). Results of the present study have indicated the shrinkage of proximal and distal tubules with the simultaneously interstitial fluid and closer of tubular lumen and necrosis of glomeruli capillaries. Significant alterations in micrometric parameters were detected. The findings consolidate the existing preview of nephrotoxicity of hexavalent chromium exposure.

Syzygium cumini pulp extract treatment in such chromium intoxicated animals has shown signs of rescue and rehabilitation of renal histology that include spaces finished, glomeruli repaired, tubules stilled swelled, draining started functional capacity. Similarly, the micrometric changes have also shown corrective measures on *Syzygium cumini* fruit pulp treatment. The unique antioxidants present in *Syzygium cumini* fruit pulp extract have been found to elevate the oxidative stress of environmental toxins on various body organs and metabolism in general (Patil *et al*., 2019). Interestingly, *Morus* treatment in the Cr intoxicated mice, the histological sections have revealed many signs of alleviations of the pathological indicators that include glomerular rehabilitation and regeneration and a decrease in the interstitial fluid indicative of the *Morus nigra* fruit extract capacity for rapid rehabilitation of the renal histology in Cr intoxicated mice.

The micrometric changes in CSA of the tubules, glomeruli and glomerular number in cortex and medulla of Cr exposure have also been found convincingly reversed in *Morus* post-treated group animals. The pathological and micrometric results show and reiterate that Cr(VI) is highly toxic to the kidneys and causes drastic pathological changes in renal histology. The most fascinating finding of this research is the discovery of rescuing potentials of *Morus nigra* fruit extract for such pathologies. This finding needs further investigation, particularly with the support of the phytochemical ingredients of *Morus nigra* fruit extract and the amelioration of biochemical and metabolic indicators in the treated animals.

The histological trends are well depicted in micrometric readings. Number of glomeruli in cortex (1.7± 0.1) as well as medullary (0.1±0.03) region is reduced as a result of chromium toxicity, while the number was retained after the treatment of *Syzygium cumini* extract (0.2±0.01) in medulla and (1.9±0.1) in cortex. It is reported that chromium exposure can reduce glomerular filtration rate (Tsai *et al*., 2017). The same trend was observed in case of cross-sectional area of glomeruli. The CSA of glomeruli in cortex region (1916.07±92.9μ²) and medullary region (2169.1±128.9μ²) reduced in Cr group and the area in cortex region (3132.5± 266.9μ²) and in medullary region (3129.1±145.2μ²) was restored near to control (2515.7±115.1μ² and 2441.7± 95.7μ²) group. The same trend was observed in case of CSA of PCT's and DCT's.

The anthocyanin and polyphenolic ingredients in the *Syzygium cumini* pulp are seemingly playing an important role in the rapid reversal of pathological signs of Cr exposure in the present research work (Selamoglu, 2017). It thus seems imperative to conduct an in-depth study to unearth the antitoxic potentials of these precious antioxidants present in *Syzygium cumini* pulp. Nevertheless, this research work indicates the importance of *Syzygium cumini* fruit pulp for renal health and its possible utilization by the common person as a renal health tonic against daily occupational and accidental exposure to environmental toxins such as hexavalent Cr in the present study.

Interestingly, *Morus* treatment in the Cr (VI) intoxicated mice, the histological sections have revealed many signs of alleviations of the pathological indicators that include glomerular rehabilitation and regeneration and a decrease in the interstitial fluid indicative of the *Morus nigra*

fruit extract capacity for rapid rehabilitation of the renal histology in Cr (VI) intoxicated mice. The micrometric changes in CSA of the tubules, glomeruli, and glomerular number in cortex and medulla of Cr (VI) exposure have also been found convincingly reversed in *Morus* post-treated group animals. The pathological and micrometric results show and reiterate that Cr (VI) is highly toxic to the kidneys and causes drastic pathological changes in renal histology. The most fascinating finding of this research is the discovery of rescuing potentials of *Morus nigra* fruit extract for such pathologies. This finding needs further investigation particularly with the support of the phytochemical ingredients of *Morus nigra* fruit extract and the amelioration of biochemical and metabolic indicators in the treated animals.

Micrometric indications also support histological signs. The reduced number of glomeruli in cortex and medullary region was restored by *Morus* extract treatment *i.e*. (2.2±0.1) and (2.3±0.1). Same goes for CSA parameter. Rehabilitation signs in CSA of glomerulus in cortex and medullary region were also observed as mean CSA of glomeruli in cortex was recorded as 2177.6±95.1μ², while for medulla it was 2066.8±52.7μ². The cross sectional area of proximal (661.7±13.7μ²) and distil convoluted tubule (686.85±41.68μ²) also followed the same pattern. *Morus* is reported to enhance the efficiency of kidney functions (Yilmaz *et al*., 2020).

Conclusion

Morus nigra and *Syzygium cumini* fruit extracts has been found to have regenerative and rehabilitative potentials that surely indicates the reno-protective potentials of this medicinal plant fruit against unintentional exposure to common environmental toxins such as hexavalent chromium.

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

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