Mitigations of Syzygium cummini Fruit Extract on CdCl₂ Induced Histopathological Changes in Laboratory Mouse Epididymids

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ABSTRACT
The aim of present project was to determine the harmful effects of CdCl₂ on epididymis of adult mice (Mus musculus). Histopathological changes in epididymis of mice treated with 50ppm of CdCl₂ were studied in this research work. Moreover, remedial effects of Jambul fruit extract were also studied. Study was conducted on 30 male albino mice divided into three groups (n=10) as follows: (a) Control (C); (untreated); (b) CdCl₂ {50ppm CdCl₂ as drinking water (15 days) and for natural recovery CdCl₂ free water (7days)}; (c) CdCl₂+Jambul extract (CdCl₂+J) 50ppm CdCl₂ in drinking water for 15 days followed by 0.2mLjambul extract (7days)}. All animals were dissected on day 21st for surgical removal of epididymis. The results revealed that mean tubular cross-sectional area of caput, corpus and cauda epididymis was significantly increased in CdCl₂ treated group compared to Control and Cd+J treated groups. A single peritoneal administration of soluble cadmium causes an acute hemorrhagic reaction in proximal end of caput epididymis. The lining epithelium of the proximal caput was atrophied, tubules were dilated and spermatozoa within the lumen were disintegrated.

INTRODUCTION
Cadmium (Cd) is a toxic heavy metal that occurs in natural environment and as a pollutant emanating from industrial and agricultural sources (Jarup and Akesson, 2009). Traces amount of Cadmium is present in water as well as in plants and animals (Min et al., 2002). Cadmium also occurs in rock erosion and abrasion, volcanic eruptions (Jarup et al., 2000), fossil fuels, particularly non-ferrous mining and metal industries (Friberg et al., 1986). The compound has varying degrees of solubility, absorption, and toxicity (Falks et al., 1990). In animal studies, various effects of cadmium on male reproductive system were reported as an accumulation of cadmium in reproductive organs, decreased testicular, epididymal, vas deferens and accessory glands weight, ischemia-induced necrosis in testes, accumulation of metal in spermatogenic cells, reduction in the number of germ and sertoli cells along with decreased androgens and gonadotropins (Shimada et al., 2009; Ozdemir and Dursun, 2009).Syzygium cummini (Jambul) is a perennial tropical tree of family Myrtaceae (Lanjewar et al., 2018). Different parts of the plant (bark, leaf, fruit and seed) have been widely investigated for their bioactive phytochemical constituents (Chhikara et al., 2018).
Its fruit pulp is rich of phenols, flavonoids and anthocyanins (Jebitta and Allwin., 2016). Its fruit pulp and seed have proven antioxidant and free radicals scavenging properties (Rohadi et al., 2017). In this study histopathological alterations studied in epididymis of mice treated with 50ppm of CdCl₂. As well as ameliorative properties observed with treatment of Jambul fruit pulp extract.

MATERIAL AND METHODS

Animals and Treatment:
Male albino laboratory mice (Mus musculus) were used in present research work. The mice were reared in the animal house in the University of Sargodha. 30 male animals of almost equal weight were selected randomly then labeled them with tail and back and keep weighting them with kitchen balance and later divided into three groups (n= 10).

1-Control (untreated) Group (C): This group was given regular drinking water only.

2-Cd Treated Group (CdCl₂): These animals were provided with 50ppm of CdCl₂ in drinking water for 15 days.

3-Cd and Jambul Extract Co-Treated Group (CdCl₂+J): These animals were given 50ppm Cd solution for 15 days and 0.2mL Jambul pulp extract regularly at 12hrs intervals for 7 days for comparative analysis.

Chemicals and Experimental Protocol:
Hydrated CdCl₂ crystals of the analytical grade were used to prepare the dose solution. Based upon following calculations initially 1000ppm CdCl₂ stock solution was prepared as; CdCl₂ stock solution (1000ppm) = dissolved 1.63g of CdCl₂ in 1 liter of drinking water. Dose solution (50ppm) was prepared further diluting the stock solution. 50mL stock solution added in 950 mL of water.

Extract:
Fresh Jambul fruit was purchased from market and pulp was separated from seeds. Then pulp was grinded using electric grinder and juice was obtained. After that juice was centrifuged.

Dissection, Processing, Sectioning & Photography:
On the day 21st of experiment, the animals were dissected for surgical removal of the epididymis. For histopathological studies, epididymis was fixed, dehydrated and processed. Section of 5µ on a microtome apparatus was obtained and stained using E&H stain. Histomorphological changes were evaluated using light microscope and photographed using a digital camera.

RESULTS

1-General Observations:
At the time of dissection various morphological changes were observed in only CdCl₂ treated group, which are thick and blackish blood with slow rate of flow, very less amount of fats was noticed around viscera, color of liver was pale and having white spots, spleen larger in size and lungs were reduced compared to control, testes were smaller in size and attached with scrotum and epididymis was highly reduced in size.

2-Body Weight:
Initial and final body weight of Control, CdCl₂, and Cd+J treated groups were recorded CdCl₂ treated groups resulted in highly significant (P<0.001) decrease in mean final body weight compared to CdCl₂+J treatment (Table 1).
Mitigations of Syzygium cummini Fruit Extract on CdCl$_2$ Induced Histopathological Changes

Table 1: Body weight of Control, CdCl$_2$, and Cd+J treated group after 15 days treatment of cadmium and (7 days post-treatment with Jambul fruit extract in Cd+J treated group) in male adult mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.6±0.51</td>
<td>33.6±0.72</td>
</tr>
<tr>
<td>CdCl$_2$</td>
<td>28.2±0.58</td>
<td>24.0±0.71$^a$$^{***}$</td>
</tr>
<tr>
<td>Cd+J</td>
<td>27.6±0.4</td>
<td>30.6±0.51$^a$$^{b}$$^{***}$</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM {a=Control Vs treated group, b =CdCl$_2$ Vs Cd+J treated group; P<0.05*, P<0.001***}

3-Organ Weight:
Mean epididymis weights of Control, CdCl$_2$ and Cd+J treated groups are given (Table 2). One-way analysis of variance revealed that both treatments i.e. Cd and Cd+J resulted in non-significant effect on epididymis weight compared to control. No significant difference in epididymis weight was observed among groups.

Table 2: Effect of 15 days treatment of 50ppm CdCl$_2$ and Cd+J(Post treated with Jambul extract for 7 days) on mean epididymis weight of adult mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Epididymis Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.26±0.07</td>
</tr>
<tr>
<td>CdCl$_2$</td>
<td>0.13±0.04</td>
</tr>
<tr>
<td>CdCl$_2$+J</td>
<td>0.19±0.05</td>
</tr>
</tbody>
</table>

Histological Observation:
Microscopic observation of the slides of sections from caput of epididymis of control mice showed tubules of regular diameter with compactly arranged interstitium. The tubular epithelium exhibited tall columnar principal cells having large stereocilia on their adluminal end (Figs. 1 & 2A). The lumen was not very wide but having lot of sperms. Cd treated sections of caput epididymis revealed the irregular placed tubules and wider interstitium with loose connective tissues (figure 1 &2B) The epithelium was made up of columnar cells less height than control, having reduced cilia and the lumen was wider having sperm along with cellular debris. In Cd+J treated caput epididymis; the tubules resemble to control but widening of interstitium reflects toxicity of metal the epithelium showed increased height of principal cells compared to Cd treated. Lumen shows the effect of cd exposer being wide and having sperms along with debris. The protective role of J extract is obvious but not very précised (Figs. 1 & 2B).
Sections of corpus epididymis belonging to control mice showed compact arrangement of tubules in narrow interstitium. Epithelium was made up of cuboidal principal cells enclosing wide lumen full of healthy sperms (Fig. 3A) while Cd treated corpus sections showed the adverse effect of metal on interstitium being wide with loose connective tissue. Tubular epithelium was made up of principal cells, lumen wide, hollow and having sperms with cellular debris (Fig. 3B). In Cd post-treated J extract corpus epididymis tubules of regular size in compactly arranged interstitium were observed (Fig. 3C) the tubular epithelium was organized made up of cuboidal principal cells,
lumen not very wide but having healthy sperms in it (Fig. 4).

The microscopic study of sections from cauda epididymis of control showed tubules of regular size arrange in wider interstitium full of connective tissues. Principal cells of epithelium were cuboidal, lumen wide full of healthy sperms (Figs. 5A & 6A). Cd treated cauda epididymis sections showed irregular interstitium with less tissue distorted tubules, the principal cells were reduced in size due to which lumen space increase having sperms and debris (Figs. 5B & 6B). The histological observation of Cd+J cauda epididymis reveals protective role of J extract on the interstitial tissues by increasing its strength but the effect of metal on epithelial principal cells was profound by decreasing their size and lumen was wide full of sperms (Figs. 5C & 6C).

**Tubular CSA of Caput Epididymis:**

Mean Tubular CSA of Caput epididymis in control, Cd and Cd+J treated group is given (Fig 1). Highest CSA of Cd group (10501.88±3523.83μ) was followed by Cd+J group (8869.02±3075.51μ) and least mean caput tubular CSA was observed in the control group (8839.07±4022.01μ). Statistical analysis (one-way ANOVA) has shown no significant variation (0.031, p>0.001).

**Epithelial height of Caput epididymis:**

Mean epithelial height of Caput epididymis in control, Cd and Cd+J treated groups are given in figure 2. Highest Epithelial height of control group (26.31±5.71μ) was followed by Cd+J group (17.277±12.08μ) and least mean epithelial height of Caput of observed in Cd group (15.98±8.41μ) Statistical analysis (one way ANOVA) has shown significant variation(.000, p>0.001).
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**Tubular CSA of Corpus Epididymis:**
Mean tubular CSA of corpus epididymis in control, Cd and Cd+J treated groups are given in fig 3. Highest Tubular CSA of corpus epididymis of Cd group (22364.19±12044.30µ) was followed by control group (11196.23±4674.77µ) and least mean Tubular CSA of corpus observed in Cd+J group (10913.13±2671.91µ). Statistical analysis (one way ANOVA) has shown significant variation (.000, p>0.001).

**Epithelial height of Corpus Epididymis:**
Mean epithelial height of corpus epididymis in control, Cd and Cd+J treated groups are given in figure 4. Highest epithelial height of control group (11.79±10.667µ) was followed by Cd+J group (13.47±5.91µ) and least mean Epithelial height of corpus was observed in control group (22.68±4.78µ). Statistical analysis (one way ANOVA) has shown significant variation (.000, p>0.001).
Fig. 4: Photomicrographs from the representing sections showing epithelium of Corpus of control epididymis (A) Corpus of Cd treated epididymis (B) Corpus of Cd+J treated epididymis (C) group [EP: tubular epithelium of caput epididymis, P: Principal cuboidal cells]; H&E staining at 1000X.

**Tubular CSA of Cauda Epididymis:**
Mean CSA of Cauda epididymis in control, Cd and Cd+J treated group is given in figure 5. Highest CSA of Cd group (26588.03±7854.02µ) was followed by Cd+J group (13898.15±3959.68µ) and least mean Tubular CSA of Cauda was observed in control group. Statistical analysis (one way ANOVA) has shown significant variation (.000, p>0.001).

Fig. 5: Photomicrographs from the representing sections showing tubules of Cauda of control epididymis (A) Cauda of Cd treated epididymis (B) Cauda of Cd+J treated epididymis (C) group [EP: tubular epithelium of caput epididymis, P: Principal cuboidal cells]; H&E staining at 400X.

**Epithelial Height of Cauda Epididymis:**
Mean epithelial height of Cauda epididymis in control, Cd and Cd+J treated group is given in fig 6. Highest epithelial height of control group (17.3093±3.20µ) was followed by Cd group (16.8012±9.59µ) and least epithelial height of Cauda of observed in Cd+J group (13.822±7.97µ). Statistical analysis (one-way ANOVA) has shown no significant variation (.044, p>0.001).
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Fig. 6: Photomicrographs from the representing sections showing epithelium of Cauda of control epididymis (A) Cauda of Cd treated epididymis (B) Cauda of Cd+J treated epididymis (C)group [EP: tubular epithelium of caput epididymis]. H&E staining at 1000X.

Table 3: Effect of 15 days treatment of 50ppm CdCl2 and Cd+J(Post treated with Jambul extract for 7 days) on Micrometeric Parameters (Values are expressed as Mean±SEM).

<table>
<thead>
<tr>
<th>Micrometeric Parameters</th>
<th>Control</th>
<th>CdCl2</th>
<th>CdCl2 + J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular CSA of Caput epididymis (µm2)</td>
<td>8839.07±4022.01</td>
<td>10501.88±3523.83</td>
<td>8869.02±3075.51</td>
</tr>
<tr>
<td>Epithelial height of Caput epididymis (µ2)</td>
<td>26.3±5.71</td>
<td>15.98±8.41</td>
<td>17.27±12.08</td>
</tr>
<tr>
<td>Tubular CSA of corpus epididymis (µ2)</td>
<td>11196.23±4674.77</td>
<td>22364.19±12044.30</td>
<td>10913.13±2671.91</td>
</tr>
<tr>
<td>Epithelial height of corpus epididymis (µ2)</td>
<td>22.68±4.78</td>
<td>11.79±10.667</td>
<td>13.47±5.91</td>
</tr>
<tr>
<td>Tubular CSA of Cauda epididymis (µ2)</td>
<td>7761.26±2968.26</td>
<td>26588.03±7854.02</td>
<td>13898.15±3959.68</td>
</tr>
<tr>
<td>Epithelial height of Cauda epididymis (µ2)</td>
<td>17.3093±3.20</td>
<td>16.8012±4.59</td>
<td>13.822±7.97</td>
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<td>13898.15±3959.68</td>
<td>26588.03±7854.02</td>
<td>13898.15±3959.68</td>
</tr>
</tbody>
</table>

DISCUSSION

Air pollution, smoking and contaminated food are major sources of cadmium exposure for general population (Satarug et al., 2010). Various studies have shown that Cadmium is reported as a reproductive toxicant in animals as well as humans (Cheng et al., 2011). Jambul (Syzygium cumini) is a common medicinal food plant of the family Myrtaceae (Sultana et al., 2007). Its fruit pulp has excellent antioxidant properties (Prochazkova et al., 2011) because it includes quite large amounts of hydrolysable tannins, flavonols, and pro anthocyanidins (Gordon et al., 2011). In silica-intoxicated rats, jambul extracts significantly reduced a general increase in the serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glucose, protein, and cholesterol (Saxena and Singh 2011).

In mice, hexane extracts (15mg/kg) of the flower buds of Syzygium aromaticum(a related species) improved testicular functions, enhanced production of steroid dehydrogenase, alcohol oxidoreductases and serum levels of testosterone in mice (Mishra and Singh, 2008). Post-treatment with Jambul fruit extract after sodium fluoride intoxication was proved to revitalize steroidogenesis and spermatogenesis in male mice (Ahmed et al., 2012). These studies showed protective ability of Jambul extract against various environmental toxicants.

Present study was designed to evaluate the protective potentials of Jambul extract against cadmium toxicity to part of male reproductive excurrent duct epididymis. The epididymis, a steroid dependent organ, is responsible for post testicular
maturation and storage of sperm because of the composition of the sperm plasma membrane and absence of cytoplasm, sperm in epididymis are susceptible to damage from reactive oxygen species (Agarwal et al., 2014). Epididymis is protected from oxidative damage by secretion of antioxidant enzymes under steroid regulation (Schwaab et al., 1998). In present study, weight of epididymis was found not to be significantly affected by the given dose (50ppm) of CdCl$_2$ for 15 days in both Cd and Cd+J treated groups compared to control. Single dose (1.2mg/kg b.wt.) of Cadmium chloride administered once to male Wister rats resulted in significant reduction in epididymis weight in one group after 7 days and in other groups after 56 days (De-souzaPredes et al., 2010). In rats treated subcutaneously with 2 mg Cd/kg body mass/day for 2 wk caused significant decrease in (60% lower) weight of the epididymis than in control animals (Herak-Kramberger et al., 2000). Seven days after the treatment of 1 mg CdCl$_2$, the weights of the caput and cauda epididymis were not affected; however, significant weight loss was that of the cauda epididymis but not that of the caput epididymis which was recorded 15 days after treatment of 1mg CdCl$_2$ administration. These studies reported variable data regarding effect of various doses of CdCl$_2$ for variable duration on weight of epididymis, while the results of present study showed that CdCl$_2$ (50ppm) for 15 days could not affect the organ weight significantly. In current experiment, mean tubular cross-sectional area of caput, corpus and cauda epididymis was significantly increased in CdCl$_2$ treated group compared to Control and Cd+J treated groups. A single peritoneal administration of soluble cadmium causes an acute haemorrhagic reaction in proximal end of caput epididymis. The lining epithelium of the proximal caput was atrophied, the tubules were dilated and spermatozoa within the lumen were disintegrated. Hoey (1966) documented accumulation of heavy metals (sc injection as soluble salts) in testicular and epididymal sperm, epithelium of excurrent ducts and surrounding connective as well as vascular tissue in rats. The increased tubular CSA due to cadmium treatment in this experiment might be the tubular dilation due to metal accumulation. While the mean tubular cross-sectional area in all parts of epididymis was found to be normalized in jambul extract post-treated group pointing towards protective abilities of Jambul against cadmium induced toxicity in various parts of epididymis.

**Conclusion**

Our results exposed the adverse effect of CdCl$_2$ (50ppm for two week) to animal epididymis weight and histopathological effects on epididymis. Furthermore, defensive role of Jambul fruit extract as reversal was discerned in all histopathology parameters of epididymis in fruit extract post-treated group against Cd induced toxicity to this reproductive duct. Our study also supports the use of natural fruit and their extracts particularly Jambul (*Syzygium cumini*) for the protection from the harmful effects of environmental toxicants especially with reference to reproductive abilities to which humans are exposed.

**REFERENCES**


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