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Original Article

Ameliorative effects of morel mushroom (*Morchella esculenta*) against Cadmium-induced reproductive toxicity in adult male rats

Efeitos benéficos do cogumelo morel (*Morchella esculenta*) contra a toxicidade reprodutiva induzida por cádmio em ratos machos adultos

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Abstract

Cadmium (Cd) is one of the major toxicants, which affects human health through occupational and environmental exposure. In the current study, we evaluated the protective effects of morel mushrooms against Cd-induced reproductive damages in rats. For this purpose, 30 male rats were divided into 6 groups (n=5/group), the first group served as the control group, second group was treated with an intraperitoneal (i.p) injection of 1 mg/kg/day of Cd. Third and fourth groups were co-treated with 1 mg/kg/day of Cd (i.p) and 10 and 20 mg/kg/day of morel mushroom extract (orally) respectively. The final 2 groups received oral gavage of 10 and 20 mg/kg/day of morel mushroom extract alone. After treatment for 17 days, the animals were euthanized, and testes and epididymis were dissected out. One testis and epididymis of each animal were processed for histology, while the other testis and epididymis were used for daily sperm production (DSP) and comet assay. Our results showed that Cd and morel mushrooms have no effect on animal weight, but Cd significantly decreases the DSP count and damages the heritable DNA which is reversed in co-treatment groups. Similarly, the histopathological results of testes and epididymis show that morel mushrooms control the damage to these tissues. Whereas the morel mushroom extract alone could enhance the production of testosterone. These results conclude that morel mushrooms not only control the damage done by Cd, but it could also be used as a protection mechanism for heritable DNA damage.

Keywords: cadmium toxicity, *Morchella esculenta*, herbal medicine, DNA damage, spermatogenesis.

Resumo

O cádmio (Cd) é um dos principais tóxicos, que afeta a saúde humana por meio da exposição ocupacional e ambiental. No presente estudo, avaliamos os efeitos protetores dos cogumelos morel contra os danos reprodutivos induzidos pelo Cd em ratos. Para tanto, 30 ratos machos foram divididos em 6 grupos (n = 5 / grupo); o primeiro grupo serviu de controle, o segundo grupo foi tratado com injeção intraperitoneal (i.p) de 1 mg / kg / dia de Cd. O terceiro e o quarto grupos foram cotratados com 1 mg / kg / dia de Cd (i.p) e 10 e 20 mg / kg / dia de extrato de cogumelo morel (por via oral), respectivamente. Os dois grupos finais receberam gavagem oral de 10 e 20 mg / kg / dia de extrato de cogumelo morel sozinho. Após o tratamento por 17 dias, os animais foram sacrificados e os testículos e o epidídimo foram dissecados. Um testículo e epidídimo de cada animal foram processados para histologia, enquanto o outro testículo e epidídimo foram usados para produção diária de esperma (DSP) e ensaio cometa. Nossos resultados mostraram que os cogumelos Cd e morel não têm efeito sobre o peso do animal, mas o Cd diminui significativamente a contagem de DSP e danifica o DNA hereditário, que é revertido em grupos de cotratamento. Da mesma forma, os resultados histopatológicos dos testículos e do epidídimo mostram que os cogumelos morel controlam os danos a esses tecidos. Considerando que o extrato de cogumelo morel sozinho pode aumentar a produção de testosterona. Esses resultados concluem que os cogumelos morel não apenas controlam os danos causados pelo Cd, mas também podem ser usados como um mecanismo de proteção para danos hereditários ao DNA.

Palavras-chave: toxicidade de cádmio, *Morchella esculenta*, fitoterapia, dano ao DNA, espermatogênese.

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1. Introduction

Heavy metal pollution is a challenging and ill-posed problem affecting the cellular physiology of living organisms (Cheng et al., 2019; Islam et al., 2018; Qing et al., 2015; Zhuang et al., 2009). The modern world depends on industrialization to meet the demand for different daily used products, which results in the production of different toxicants as by-products. Mostly these by-product toxicants are disposed to environment. Among theses One important by-product toxicant is Cadmium (Cd), featured by its solubility in water, transferability, persistence and durability, universality, and severe toxicity (Li et al., 2015). Because of these properties, Cd is reported as the sixth most dangerous chemical for living things by the Agency of Toxic substances and Disease Registry (ATSDR) (Li et al., 2018; Ramelli et al., 2009; Zhang et al., 2017). Despite these hazardous features of Cd and warning of ATSDR, the world health organization (WHO) reported that the quantity of Cd is increasing with human activities and accumulate in the air, soil, and water (WHO, 1960; Krzyzanowski and Cohen, 2008; Ramelli et al., 2009; Trejo et al., 2016) resulting in its adsorption by plants, which is diet source for human and animals (Rafati Rahimzadeh et al., 2017; Faroon et al., 2012; Järup and Åkesson, 2009; Ramelli et al., 2009). With respect to food intake, in plant food sources Cd is found in cereals (wheat and rice), root vegetables (potato, celeriac, carrot), and green leafy vegetables, while in animals it is found in cephalopods, crabs, molluscs, crustaceans, and offal products of old animals. It is reported that food from plant sources has a higher concentration of Cd than dairy and poultry products (Järup and Åkesson, 2009).

Recent studies have reported diverse toxic effects of Cd, including teratogenicity, oncogenicity, renal dysfunction, endocrine disruption, and reproductive toxicity (Bernard, 2008; Zhang et al., 2017). According to some epidemiological studies, Cd and lead (Pb) have an equivocal effect on hormones concentration, sperm parameters, and male infertility (Benoff et al., 2000). In addition to environmental toxicants, climatic seasons and geographical locations greatly affect reproductive physiology (Becker and Berhane, 1997; Fisch and Goluboff, 1996; Paulsen et al., 1996; Šrám et al., 1996). But enough data is available which demonstrate that high concentration of heavy metals like Cd in the environment are associated with low semen quality (Danielsson et al., 1984; Oldereid et al., 1993; Ragan and Mast, 1990). The exact mechanism of Cd toxicity is not understood completely till now, but in the past few decades, different studies have reported major cellular toxicities including oxidative stress (Casalino et al., 2002; Hussain et al., 1987; Shukla et al., 1987), variation in thiol proteins (Chan and Cherian, 1992; Li et al., 1993), inhibition of mitochondrial activity (Müller, 1986), variation in membrane structure and function (Müller, 1986; Shukla et al., 1987), damage to DNA structure (Coogan et al., 1992), expression of stress gene (Goering et al., 1993; Wang and Templeton, 1998) and variation in some enzymatic activities (Casalino et al., 2002, 2000, 1997; Jay et al., 1991; Manca et al., 1991; Wätjen et al., 2001)

For centuries Morchella Esculenta (ME) along with other species of Morchella has been used in Traditional Chinese Medicine (TCM) because of its active pharmacological constituents (Baati et al., 2011; Duncan et al., 2002; Halliwell, 2012, 2011; Heleno et al., 2013; Meng et al., 2010; Raman, 2018). It is also used as medicine in Japan, Malaysia, India, and Pakistan for its aphrodisiac properties (Gewali, 2009; Raman, 2018; Sud and Sud, 2017). It is reported that the fruiting body of ME contains profound antitumor, anti-inflammatory, and antioxidant activity (Elmastas et al., 2006; Nitha et al., 2010; Nitha and Janardhanan, 2008). To the best of our knowledge, no scientific data regarding its role in reproductive physiology is known.

Based on damages caused by Cd, and the presence of bioactive elements in ME, which could be used in controlling the oxidative in living organisms, the current study is designed to check the effects of Cadmium Chloride ($CdCl_2$) administration on reproductive parameters of male rats and whether ME can reverse these effects.

2. Materials and Methods

2.1. Animals

Thirty adult male Sprague Dawley (SD) rats, having an average weight of 260 ± 45 grams, were obtained from animal house of College of Animal Sciences and Veterinary Medicine Jilin University Changchun, and were kept in stainless steel cages according to standard guidelines at controlled temperature (24 ± 2°C) and humidity (50-60%) for one week to acclimatize to lab environment. All the animals were kept at 12h/12h dark/light cycle and were fed with standard feed and had free access to water ad libitum. The whole experiment was approved by the ethical committee of Jilin University Changchun China (Permit Number SY201909012)

2.2. Chemicals

Cadmium chloride ($CdCl_2$) was purchased from BDH Chemicals Ltd (pool, England)

2.3. Collection of ME and preparation of its extract

Morel Mushrooms (*Marchella esculenta*) (wild) was obtained from different fields of District Swat Pakistan. It was verified as Morel Mushrooms at the department of Plant sciences Quaid-i-Azam University Islamabad. After verification, the mushrooms were dried under shade and stored in humid free environment.

Ten days before the animal trials started the ME was weighted and mixed with ethanol. The ratio of dried ME and ethanol was roughly 1:3 in bottle. This mixture was on magnetic stirrer for one week.After one week this mixture was filtered using filter paper . The solvent was evaporated, and the remaining extract was weighed and used for further study.

2.4. Experimental design

Adult male rats were divided into six groups (n=5/group). The first group served as Control group and received

intraperitoneal (i.p) injection of saline, three groups were treated with an i.p. injection of 1 mg/kg/day of Cd using $CdCl_2$ solution. Among these 3 groups, 2 groups were co-treated with oral gavage of ME extract (one group was co-treated with 10mg/kg/day and the other with 20mg/kg/day of ME extract along with Cd). The remaining 2 groups received 10 and 20 mg/kg of ME extract alone respectively using an oral gavage (Figure 1). The exposure of Cd was for 17 days according to ATSDR, Cilenk, and our previous work (Çilenk et al., 2016; Iqbal et al., 2021; Ramelli et al., 2009). On day 18 (roughly 24 hours after last dose administration) the animals were anesthetized and euthanized according to guidelines of Jilin University. Blood plasma was collected for hormonal analysis, while reproductive organs (Testes and Epididymis) were dissected out and processed for tissue and sperm analysis. One of the testis and epididymis of each animal were fixed in 10% Formaldehyde for histological analysis, while the others were stored at -70°C until further analysis.

Before storing or processing the organs, the volume of testes was measured in measuring cylinder using saline (37°C) as measuring liquid.

2.5. Daily sperm production (DSP)

The testes stored at -70°C were defrosted and used to calculate DSP according to Robb et al.(Robb et al., 1978) with some modifications. Briefly, testes were weighted, tunica albuginea were removed and 90 mg of tissue was homogenized in 2ml of saline and diluted according to Jahan et al.(Jahan et al., 2016), then 5.5µl of the sample was taken on Neubauer chamber (haemoctmeter), covered with a small coverslip, and late sperm cells were counted under a microscope at 40X magnification.. The DSP was counted using Formula 1

Formula for DSP

$$Y = \left(\frac{x}{16}\right) \times 100 \times 5 \times 5.5 \times 1000 \quad (1)$$

Where Y is the total number of spermatids, x is the number of spermatids counted on hemocytometer. 16 is the total number of squares observed, 100 is the total number of squares, 5 is dilution made, 5.5 µl was loaded on the haemocytometer, while 1000 is to convert µl into ml.

2.6. Histology

One testis and epididymis of each animal were fixed in 10% formaldehyde and dehydrated using different concentrations of ethanol before embedding in paraffin wax. Then 5-7µm thick sections were cut from the prepared paraffin blocks using a microtome. Sections were affixed on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E) stains. These slides were examined using microscope equipped with the micro-photographic system. Different parameters (diameter of seminiferous tubules, interstitial space, diameter of tubular lumen, tunica albuginea, and height of the epithelium) of seminiferous tubules were measured in the slides using image J software.

2.7. Quantitative determination of Testosterone concentration

Testosterone concentration in blood plasma was determined using Enzyme Linked Immuno Sorbant Assay (ELISA) kit. The ELISA kit was purchased from Amgenix, Burlingame, CA, USA. All samples were quantified in duplicate in a single assay.

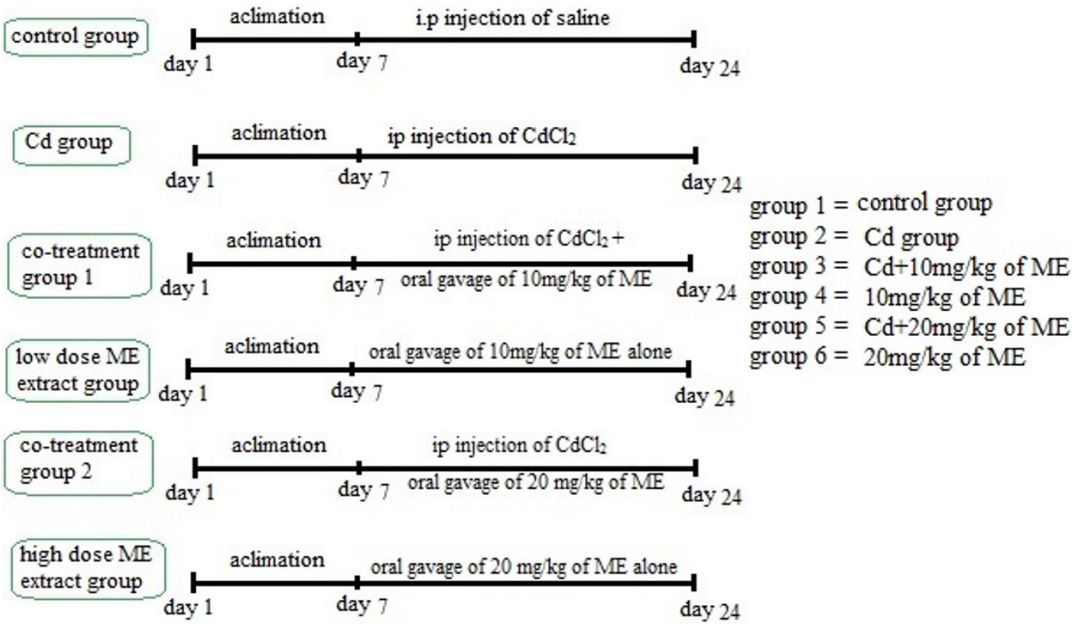

Figure 1. experimental design showing that the control group is treated with ip dosage of saline, 3 groups are treated with ip dosage of Cd, in which 2 received oral dosage of ME, and final 2 groups received oral gavage of ME alone (ME= *Morchella esculenta*).

2.8. Comet assay (single-cell gel electrophoresis (SCGE))

The DNA damage in sperm cells was determined using comet assay with some modifications (Ahmad et al., 2007). Briefly 100 µl of 1% regular melting point agarose was placed on glass slides and allowed to solidify. After that 20 µl of homogenate of cauda epididymis and 65 µl of low melting point- agarose were mixed and spread on the agarose-coated slides. After solidification, slides were submerged in lysing solution (100 mM EDTA Na2, 10mM Tris, 2.5M NaCl, pH 10, 1% triton X 100) overnight in dark (so that direct light doesn't produce more DNA damage). In the final step of processing a horizontal gel electrophoresis tank was filled with electrophoresis solution (300mM NaOH, and 1mM EDTA, pH 12.5), and slides were placed in it with agarose end facing toward the positive terminal. The DNA fragments were separated by electrophoresis for 10 min at 300 mA and 25 V.

For fluorescent microscopy, 100-200 ml of 20mg/ml acridine orange solution was overlayed by using a coverslip. The comets were analyzed using Casplab_1.2.3b2.

2.9. Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test was applied to all the experimental data for comparison of different groups by using Graphpad prism software. All the results are shown as mean ± SEM. The significance level was set at $p < 0.05$.

3. Results

3.1. Final weight of animals and reproductive organs

The mean final weight of animals in the control and all experimental groups, and the weight of testis, epididymis and volume of testes showed no significant variation (Table 1)

3.2. Daily Sperm Production (DSP/T x 10^6)

The mean value of daily sperm production in all the experimental groups showed significant change. The number of sperm in Cd treated group was significantly reduced ($p < 0.001$), while non-significant increase was seen in co-treatment groups as compared to Cd group (Table 1). This indicates that ME plays a role in preventing (controlling) the damage caused by Cd to DSP. The number of sperm in the ME treated animals are significantly higher than the Cd treated group, but interestingly the results shows a slight decrease (non-significant) in DSP of ME treated group as compared to the Control. (Table 1)

3.3. Protective effect of Morel Mushrooms against Cd induced Damage to heritable DNA

Anomalies in the DNA of cauda epididymal sperms were measured by comet assay, the mean value of comet head length in Cd treated group showed significant ($p < 0,01$) reduction when compared to the control group while the remaining parameters showed no significant variations from the control group (table 2, figure 2b)in both the cotreatment groups, no significant variations were noticed in head length of comet and percentage of DNA in head or tail (table 2). But the tail length was reduced significantly in both groups as compared to the control group ($p < 0.05$ for Cd + 10 mg/kg extract and $p < 0.01$ for Cd + 20 mg/kg extract) (table 2, figure 2C &D). the 10 and 20mg/kg extract treated groups showed significant variation from the remaining groups. The head length in 10 mg extract alone group was significantly increased from Cd treated ($p<0.001$) and Cd +10mg extract treated ($p<0.05$) groups (table 2). The tail length in both the extract alone treated groups was significantly ($p<0.01$) reduced than that in the control group, while the quantity of DNA in head was significantly increased in both these groups as compared to the control and Cd treated groups, while the tail moment was also significantly reduced in both these groups when compared to the control group (figure 1, table 2)

Table 1. Mean ± SEM of I initial and final weight of rats, its testes and epididymis, volume of testis, and daily sperm production after treatment.

Groups		Control	Cd	Cd+10 mg/kg extract	Cd + 20 mg/kg Extract	10 mg/kg extract alone	20 mg/kg extract alone
Weight (gm)	initial	272.4 ±4.15	295 ±6.12	272.5±11.1	295 ± 12.58	285.25 ± 3.68	271.25 ±6.57
	final	288.4 ±4.77	318.5 ±8.91	281.5±12.8	306 ± 44.84	308.5 ± 3.22	297.5 ±3.86
weight of testis (gm)	right	1.29 ± 0.03	1.19 ±0.16	1.05 ± 0.17	1.08 ±0.11	1.28 ± 0.03	1.19 ± 0.3
	left	1.28 ± 0.03	1.15 ± 0.17	0.86 ±0.24	1.26 ± 0.11	1.31 ± 0.04	1.13 ± 0.14
Volume of testis (ml^3)	right	1.5 ±0.2	1.52 ± 0.26	1.12 ±o.29	1.25 ± 0.22	1.7 ± 0.0.8	1.75 ±0.08
	left	1.5 ±0.2	1.37 ± 0.16	1.27 ±0.31	1.41 ± 0.19	1.57 ± 0.22	1.5 ±0.15
weight of epididymis	right	0.45 ±0.05	0.58 ± 0.02	0.47 ± 0.03	0.45 ±0.02	0.45 ± 0.02	0.66 ± 0.22
	left	0.44 ±0.04	0.51 ± 0.03	0.5 ± 0.03	0.48 ± 0.02	0.47 ±0.01	0.69 ± 0.23
DSP (× 10^6)		14.5 ±0.29	3.6 ± 1.6^{a***}	6.2 ± 1.3^{a*}	5.6 ± 0.8^{a***}	9.06 ±1.6^{b*}	8.1 ±0.8^{b*}

(All values are expressed as Mean ± SEM *= P<0.05, *** P < 0.001, a = comparison to control, b = comparison to Cd group).

Table 2. DNA damage as expressed by different parameters in control, Cadmium, Cd + 10 mg of extract, Cd +20 mg extract, 10 mg extract and 20 mg extract treated groups.

Parameters	Head length (μm)	Tail length (μm)	%DNA in Head	%DNA in Tail	Tail moment
Control	158.70 ± 6.43	30.65 ±1.92	90.24 ±0.72	10.84 ±3.21	3.60 ±0.53
Cadmium	114.80 ±7.35^{a**}	24.96 ±2.35	88.94 ±1.64	10.85 ±1.33	2.76 ±0.38
CD+10mg extract	130.00 ± 7.45	19.96 ±2.29^{a*}	91.97 ±1.31	7.19 ±0.68	1.93 ±0.43
Cd + 20mg extract	139.20 ± 11.63	15.78 ± 2.137^{a**}	93.59 ±0.75^{b*}	6.45 ±0.73	1.42 ±0.42^{a*}
10 mg extract alone	171.90 ±9.74^{b***c*}	17.94 ±2.01^{a**}	94.62 ±0.50^{a**b**}	5.01 ±0.43^{b**}	1 .42 ±0.26^{a**}
20 mg extract alone	127.80 ±7.32^{e**}	15.82 ±2.10^{a***b*}	94.49 ± 0.58^{a**b**}	5.45 ± 0.48^{b**}	1.15 ±0.33^{a**b*}

(All values are expressed as Mean ± SEM) (*= P<0.05, ** P<0.01, *** P < 0.001) (a = control, b = cadmium, c = Cd+10 mg extract, d = Cd+20mg extract,e = 10 mg extract alone).

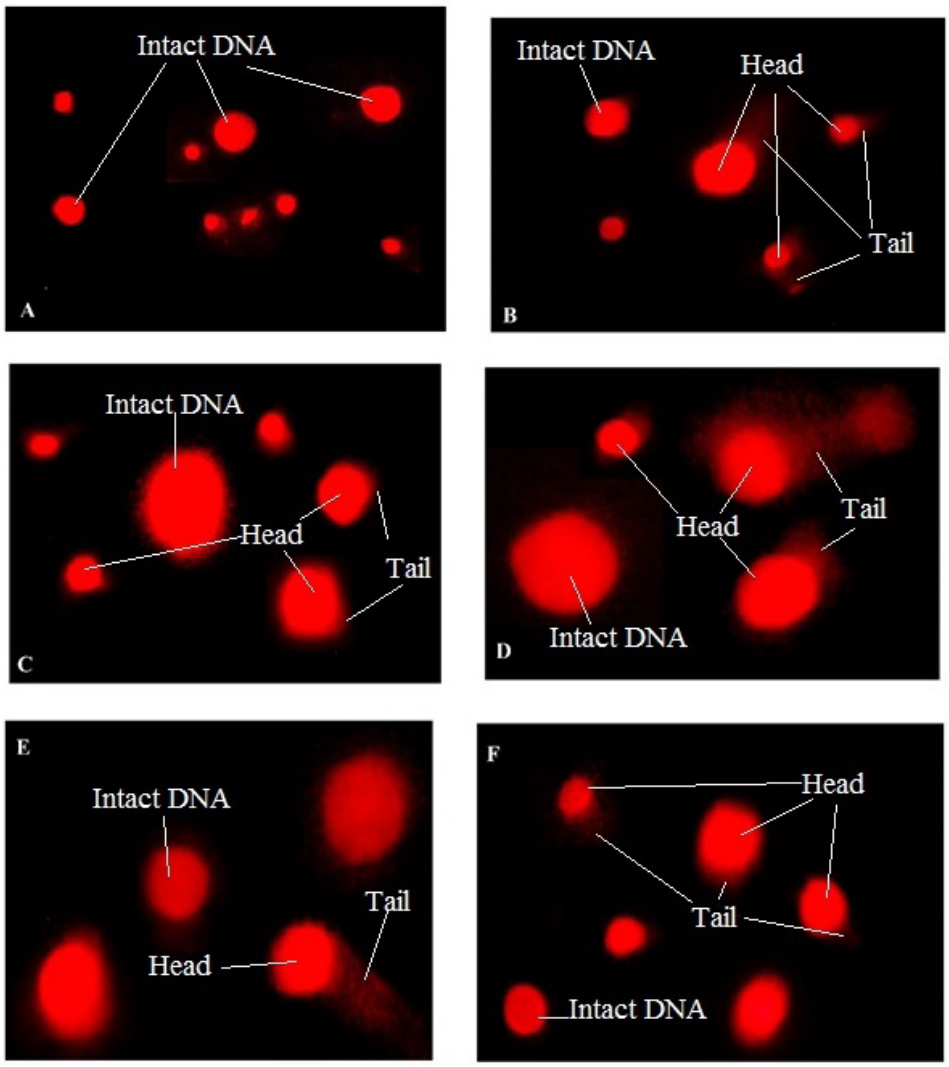

Figure 2. Fluorescent photomicrograph of sperm DNA, using comet assay, stained with acridine orange. (A) control with more intact DNA; (B) cadmium group with comets; (C) Cd + 10 mg extract group showing intact DNA with a very short tail length; (D) Cd +20 mg extract group having tail of long length and intact DNA; (E) 10 mg extract alone group in which a tail could be notice but very lesser DNA damage was found; (F) 20 mg extract alone group having short tails and intact DNA.

3.4. Protective Effect of Morel Mushroom Against Cd induced Histopathological Changes

3.4.1. Testicular tissue

Parameters studied in testicular histology include the mean length of interstitial space, height of tunica albuginea, height of epithelium and diameter of seminiferous tubules and luminal diameter. All the tubules (in all 6 groups) could be devided into different categories based on there morphological appearance. We broadly devided all the tubules into 2 categories, first showing the first half (stage 1to 8) of spermatogenesis, and 2nd showing final half of spermatogenesis (stage 9-14). Table 3 is representing the average values of all the tubules, where we observed significant variation in all 5 parameters when we compared the Cd treated group, to control group, the interstitial space and lumen of the tubules was increased significantly ($p<0.001$), while the remaining three parameters showed significant reduction (Table 3, figure 3b). In both co-treatment groups, the interstitial space was similar to the control group, but significantly ($p<0.001$) increased from the Cd treated group. similarly, the thickness of tunica albuginea and diameter of the seminiferous tubules in co-treated groups were decreased ($p<0.001$) than the control group, but significantly ($p<0.001$) increased than the Cd treated group. The change in histological parameters of the extract treated groups were similar to the co-treatment groups (figure 3 E & F, Table 3).

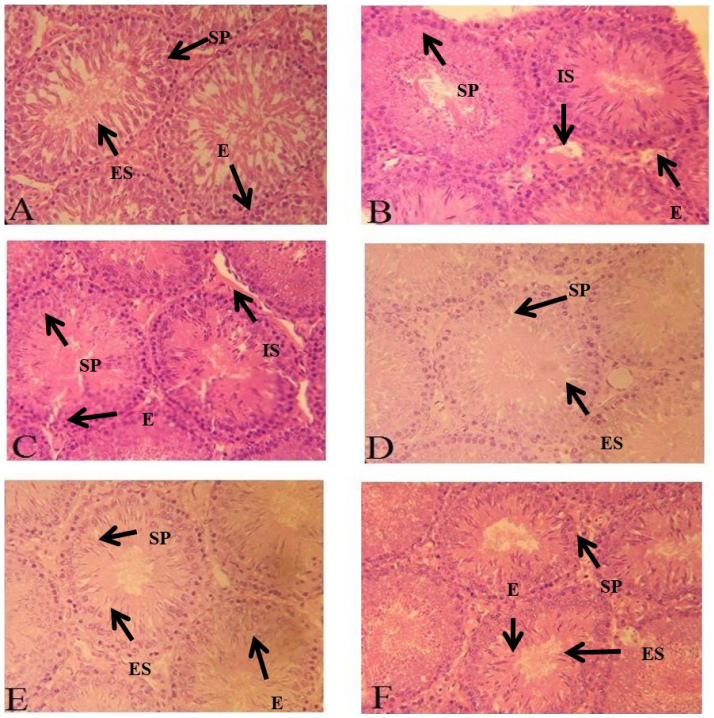

Figure 3. Photomicrograph of seminiferous tubules. (A) Control; showing compact tubules, filled lumen with spermatid, normal germ cell proliferation along epithelium; (B) Cd treated group; showing tubules with empty lumen and degenerated epithelial layer with increased interstitial space; (C) Cd+10 mg extract treated group & (D) Cd+ 20 mg extract treated group; showing minimal damage to epithelium, lumen filled with spermatid and less interstitial space; (E) 10 mg extract alone group and (F) 20 mg extract alone group; showing narrow lumen, increased epithelial height and compact tubules with less interstitial space. Magnification x40. Spermatogonia (SP), Elongated spermatids (ES), Interstitial space (IS), Epithelium (E).

Table 3. Mean ± SEM of interstitial space (µm), tunica albugenia height (µm), seminiferous tubule diameter (µm), epithelial height (µm) and tubular lumen (µm) of rat's testis in control, Cadmium, Cd + 10 mg of extract, Cd +20 mg extract, 10 mg extract and 20 mg extract treated groups.

Group	Interstitial space (µm)	Tunica albugenia height (µm)	seminiferous tubule diameter (µm)	Epithelial height (µm)	Tubular lumen diameter (µm)
Control	6.01 ± 0.44	31.13 ± 1.12	235.02 ± 5.26	69.65 ± 2.20	20.53 ± 0.81
Cadmium	13.28 ±0.80^{a***}	20.16 ± 1.26^{a***}	159.51 ± 3.68 ^{a***}	36.86 ± 2.78^{a***}	36.82 ± 2.45^{a***}
Cd + 10 mg ext	5.36 ± 0.53^{b***}	23.18 ± 0.67^{a***}	209.84 ± 4.02^{ab***}	64.25 ± 4.37^{b***}	23.03 ± 1.45^{b***}
Cd + 20 mg ext	5.16 ± 0.29 ^{b***}	22.53 ± 1.15^{a***}	185.69 ± 4.63^{abc***}	59.18 ± 3.32^{b***}	26.71 ± 1.18^{b***}
10 mg ext alone	5.78 ± 0.30 ^{b***}	28.95 ± 1.08^{b***cd**}	203.55 ± 3.14^{ab***d*}	58.65 ± 1.92^{b***}	24.43 ± 1.85^{b***}
20 mg ext alone	5.26 ± 0.41 ^{b***}	24.63 ± 1.51^{a**}	208.67 ± 4.07^{ab***d**}	53.25 ± 2.83^{ab**}	28.46 ± 1.43^{ab**}

(All values are expressed as Mean ± SEM) (*= P<0.05, ** P<0.01, *** P < 0.001) (a = control, b = cadmium, c = Cd+10 mg extract, d = Cd+20mg extract, e = 10 mg extract alone).

3.4.2. Epididymis

The histological analysis of caput and cauda epididymis showed that the ductular diameter along with its lumen diameter and height of the epithelium in the Cd treated group was significantly reduced as compared to all the remaining groups (figure 4). The co-treatment groups showed reduction in diameter of ducts and its lumen in comparison to the control, but non-significantly increase than the Cd treated group. (figure 4)

3.5 Effect of Cd and ME on blood plasma Testosterone level

The plasma testosterone level was reduced significantly ($p<0.001$) in Cd treated group as compared to the Control Group. Whereas in co-treatment groups, a decrease was noticed but only Cd + 20 mg group showed significant $p<0.05$) change when comparison was made with control. The testosterone level in both co-treated groups was significantly ($p<0.001$) higher that Cd group. While the testosterone level in both the extract alone groups was slightly higher that the control group, but the difference was not significant (Figure 5).

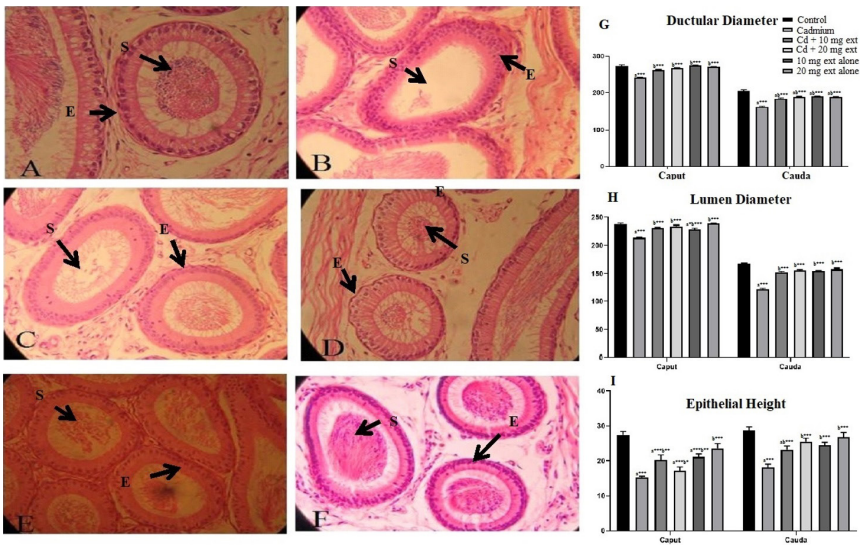

Figure 4. photomicrograph of cross section of epididymis (cauda) of rats (H&E, 40X) from: (A) Control group; showing normal morphology of cauda epididymis showing compactly arranged tubules with thick epithelium, lumen filled with sperm; (B) Cadmium group; showing marked changes in structure of tubule with decreased concentration of sperm; (C) Cd+10 mg extract group & (D) Cd+ 20 mg extract group; showing regular arrangement of tubules surrounded by stroma, lumen filled with spermatozoa; (E) 10 mg extract alone group and (F) 20 mg extract alone group ; showing increase in epithelium an lumen sperm concentration. Spermatozoa (S), Epithelium (E), Stroma (St). G, H and I summarizes the variations in tubule and lumen diameter and height of epithelium.

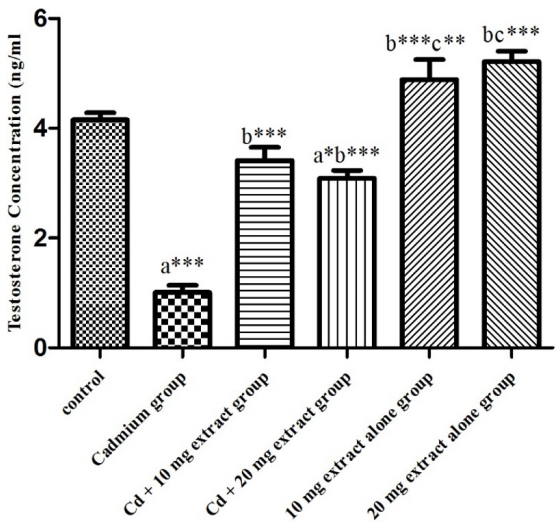

Figure 5. Mean ± SEM of plasma testosterone (ng/ml) concentration in rats of control, Cadmium, Cd + 10 mg of extract, Cd +20 mg extract, 10 mg extract and 20 mg extract group. (All values are expressed as Mean ± SEM) (*= P<0.05, ** P<0.01, *** P < 0.001, a=control, b= Cadmium, c= Cd+10 mg extract).

4. Discussion

Edible Mushrooms are the source of food and medicine for centuries (Wasser, 2002). These are considered as rich source of proteins and carbohydrates (Yun and Hall, 2004). In preterit past, the water-soluble contents in mushrooms were used as medicine. Specifically, the polysaccharides in mushrooms are reported to have immunomodulating and antitumor features (Borchers et al., 1999; Wasser, 2002). The fruiting body of ME is rich in carbohydrates, protein, vitamins (B, A, C and D) and minerals, while low in calories and fat content (Mattila et al., 2001; Negi, 2006). The ME along with its dietary and pharmacological properties is also reported to be widely used for its aphrodisiac properties (Gewali, 2009; Sud and Sud, 2017). On the other side Cd is reported with opposite properties. In remote past It is suggested that different cells and pathways, are interfered by Cd particularly the pituitary-hypothalamus-gonadal pathway (Bertin and Averbeck, 2006; Cheng et al., 2019; Waisberg et al., 2003). The cell proliferation, cell cycle progression, differentiation, the process of DNA repair and replication and the apoptotic pathways are all impaired (Cheng et al., 2019; Fang et al., 2002; Oh and Lim, 2006; Yang et al., 2004).

In previous literature, contradictory results have been reported about the effect of Cd on weight of organisms, where some articles had reported that Cd has negative effect on weight gain of organisms (Lynch et al., 1976; Ren et al., 2019; Asagba et al., 2007), but our findings are in accordance with Bebe and Panemangalore (1996), as there was no significant difference observed in weight gain of animals treated with Cd and the control group (Table 1). While looking at the nutritious components of ME (Halliwell, 2012; Raman, 2018) low calories and fat content, we observed no significant difference in weight gain of all ME extract treated groups from the control group.

In current study, a vital Cd generated reproductive damage in Cd treated group was observed, but the weight of testis, epididymis and volume of testis was almost similar in all the groups. In some previous studies (El-demerdash et al., 2004; Asagba et al., 2007; Santos et al., 2006) a huge impact of Cd is reported on reproductive and accessory sex organs along with weight of animals, although some studies suggest no or very less change on accessory organs (Predes et al., 2010; Wade et al., 2002). These structural discrepancies are directly related to physiological problems(Sinha Hikim et al., 1988). To determine the protective effect of ME against the morphological damage caused by Cd, histopathology of testis and epididymis was performed and clear deformities in the seminiferous tubules of Cd treated animals were noted. Similar results were reported previously in case of reproductive toxicity caused by Cd (Adamkovicova et al., 2014; Afsar et al., 2018; Sakr and Nooh, 2013; Wang et al., 2019). The co-treatment and extract alone treated groups showed interesting results as ME is never used before as treatment for heavy metal toxicity or improving the process of spermatogenesis. The use of medicinal herb and its extracts are repeatedly reported in Ayurvedic medicine (India), Unani medicine (Pakistan) and Chinese Traditional Medicine (TCM) for different purposes, (Mishra and Singh, 2016). Cd group showed expected results on DSP as discussed in earlier studies that morphological changes are directly related to physiological changes(Pires et al., 2013). Additionally, Cd induced oxidative stress which is the vital reason of decrease in DSP (Wong and Cheng, 2011). But in Co-treatment groups and ME extract alone group the sperm count is increased significantly as compared to the Cd group. According to Pires and his team, the low level of testosterone is related to injurious effects of Cd (Pires et al., 2013). Results of current study showed that ME not only restored the damage done by CD, but also enhances the production of testosterone. And this might explain why in past people used ME as an aphrodisiac medicine.

5. Conclusion

The current study provides an evidence that ME control the damages caused by acute exposure of Cd to testicular tissue. But further study is require to select proper dose to completely control the damage. In past ME is used for its aphrodisiac properties, in current study the results of testosterone level could explain the aphrodisiac properties of ME. But further study are required in this regard.The results of this study explain the potential prospects of ME for the treatment of heavy metal toxicity.

6. Future Prospects

Based on findings of this article, the effects of ME on different reproductive system pathways including pituitary-hypothalamus-gonadal axis should be studied. This study provides basis for herbal treatment of reproductive impairments caused by heavy metals. Further studies are required to find underlying metabolic pathways and molecular mechanisms adopted by ME extract affecting reproduction.

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