Trop J Nat Prod Res, March 2021; 5(3):549-554

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of Epigallocatechin Gallate on Cadmium Chloride-Induced Changes in Behavior, Biochemical Parameters and Spermatogenesis of Male Sprague Dawley Rats

Subramani Parasuraman*, Lam C. Hui, James Y. K Beng, Brenda N. Y Qin

Faculty of Pharmacy, AIMST University, 08100 Bedong, Malaysia

ARTICLE INFO

Article history:
Received 22 January 2021
Revised 27 February 2021
Accepted 15 March 2021
Published online 01 April 2021

ABSTRACT

Epigallocatechin gallate (EGCG) is the ester of epigallocatechin and gallic acid and is a type of catechin. EGCG is abundant in dry tea leaves and its ameliorative effect on heavy metal-induced behaviour and spermatogenesis is not clear. Hence, the present study is planned to study the ameliorative effect of EGCG on cadmium chloride ($CdCl_2$)-induced changes in behaviour and spermatogenesis of *Sprague Dawley* rats. Healthy, male rats were divided into four groups *viz.*, control, $CdCl_2$ (5 mg/kg), $CdCl_2$ (5 mg/kg) + vitamin C (200 mg/kg) and $CdCl_2$ (5 mg/kg) + EGCG (50 mg/kg) respectively. All the animals were administered with the respective assigned treatment by intraperitoneal route for 28 days. During the study, body weights changes and the behavioral functions (locomotor activity, grip strength, and escape latency tendency) of the rats were monitored at weekly intervals. At the end of the study, sperm samples were collected from cauda epididymis and motility was checked. Later, the number of spermatozoa was calculated using the red blood cell counting method. The animals administered with $CdCl_2$ and $CdCl_2$ + EGCG showed a significant reduction in body weight, behavioral functions and the number of sperm cells. Vitamin C prevented $CdCl_2$-induced changes in behavioral functions and reduction in the number of sperm cells. In the present study, EGCG failed to prevent $CdCl_2$-induced changes in the behavior of the rats and reduction in the number of sperm cells whereas, vitamin C has ameliorative effects on $CdCl_2$-induced changes in behavioral functions and reduction in the number of sperm cells.

Keywords: Behavioral functions, Biochemical parameter, Cadmium, Spermatozoa.

Introduction

The main threats to human health from heavy metals are associated with exposure to lead, cadmium [Cd], mercury and arsenic. In the current era of growing technology, the concentration of heavy metals present in drinking water and beverages is still not within the recommended limits as set by the regulatory authorities in different countries of the world. Drinking water and beverages contaminated with heavy metals such as arsenic, cadmium, nickel, mercury, chromium, zinc, and lead is becoming a major health concern for the public and health care professionals. Occupational exposure to heavy metals is known to occur by the utilization of these metals in various industrial processes and/or contents including color pigments and alloys.[1]

Cadmium is one of the major toxic metals, well-known for its occupational health risks. Cadmium exists naturally in the earth's crust as minerals and are insoluble in water. However, some cadmium which is in the salt form such as cadmium chloride [$CdCl_2$], cadmium sulphate [$CdSO_4$] and cadmium nitrate [$Cd(NO_3)_2$] are soluble in water.[2] Cadmium is commonly used as electroplating in chemical products and pigments, and as stabilizers for plastics and batteries.[3] It is also of increasing concern as a pollutant of the general environment with implications for public health.

*Corresponding author. E mail: parasuphd@gmail.com
Tel: +60 108826480

Citation: Parasuraman S, Hui LC, Beng JYK, Qin BNY. Effect of Epigallocatechin Gallate on Cadmium Chloride-Induced Changes in Behavior, Biochemical Parameters and Spermatogenesis of Male Sprague Dawley Rats. Trop J Nat Prod Res. 2021; 5(3):549-554. doi.org/10.26538/tjnpr/v5i3.21

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Cadmium accumulates in the human body and may affect the kidney, liver and testis which are considered the critical target organ in cases of chronic exposure. Cadmium affects the blood-testis barrier (BTB) *via* specific signal transduction pathways and signaling molecules, such as p38 mitogen-activated protein kinase (MAPK) thereby causing testicular cancer and also disrupt the Zn^{2+} and/or Ca^{2+} mediated cellular events in testis.[4] Inhalation of cadmium fumes or dust is the main route for occupational exposure to cadmium, whereas food and water contaminated by cadmium are the predominant sources of environmental exposure to cadmium in the general population. Cadmium emissions have increased dramatically during the 20th century because those cadmium-containing products are rarely recycled but often dumped together with household waste.[5] In recent years, ubiquitous cadmium pollution has drawn great concern due to its adverse effects on the reproductive system. It has been demonstrated that low dosage of cadmium (50 µg/day) adversely affects mammalian reproductive function, with effects that include the disruption of testis and epididymis histology, damage to spermatogenesis, a decrease in sperm motility, a change in sperm morphology and a decrease in the acrosome reaction rate in rats.[6] The epididymis and vas deferens are extremely important accessary organs that play vital roles in sperm maturation and storage. It has been demonstrated that cadmium exposure in rats (2 mg Cd/kg body mass/day) led to alkalization of the lumen fluid of the epididymis and vas deferens by direct inhibition of Proton-pumping ATPase (H^+-ATPase) function.[7,8] Natural antioxidants are gaining major attention in the management of oxidative stress-induced inflammatory disorders in the cardiovascular, metabolic and reproductive systems. Tea is the most widely consumed drink in the world, and known antioxidant. Tea is prepared by boiling water over cured leaves of the *Camellia sinensis* (Theaceae) and this plant leaf contains (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, epigallocatechin-3-gallate/ epigallocatechin gallate (EGCG), (−)-epicatechin gallate, gallic acid and caffeine.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

EGCG is a polyphenol compound and rich in tea.[9] EGCG was found to have potent antioxidant activity that benefits in the treatment of disorders such as cancer and its. EGCG is a potential cancer chemopreventive agent because it has the ability to inhibit cellular oxidation.[10] EGCG has been reported to have antimutagenic, anticancer, antidiabetic, anti-inflammatory, antibacterial, antiviral, antiobesity and neuroprotective effects.[11] Cadmium exposure occurs from the ingestion of contaminated food or water and can produce long-term health effects. Long-time use of cadmium may cause neurodegeneration and organ failure. EGCG is the plant phytoconstituents commonly present in green tea and it has a significant antioxidant and neuroprotective effect. The effect of EGCG on heavy metal-induced behavior, biochemical parameters and spermatogenesis are not clear. Hence, the present study is planned to investigate the effect of EGCG on $CdCl_2$-induced changes in behavior, biochemical parameters and spermatogenesis of male *Sprague Dawley* (SD) rats.

Materials and Methods

Chemicals

Calcium chloride ($CaCl_2$), $CdCl_2$, magnesium sulfate ($MgSO_4$), monopotassium phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), sodium lactate, sodium pyruvate, potassium chloride (KCl) and vitamin C were purchased from a local supplier in Malaysia. EGCg green tea extract - 400 mg capsules were purchased from Now Foods, USA. Each capsule of EGGg contains 200 mg of EGCG.

Animals

Healthy, adult, male SD rats with a weight of 180 ± 20g, obtained from the Central animal house, AIMST University, Malaysia were used for the study. The rats were housed and maintained in a large, spacious polyacrylic cages at an ambient room temperature with a 12h light/12h dark cycle. The animals were fed with water and normal rodent pellet diet *ad libitum*. The study was approved by the AIMST University Human and Animal Ethics Committee (AUAEC/FOP/2019/11), and the study was conducted according to the Animal Research Review Panel guidelines.

Effect of EGCG on $CdCl_2$-induced changes in behavior, biochemical parameters and spermatogenesis

A total of 24 rats were used for the study. The animals were divided into four groups of six animals each as follows:

Group I : Control

Group II : $CdCl_2$ (5 mg/kg)

Group III : $CdCl_2$ (5 mg/kg) + vitamin C (200 mg/kg)

Group VI : $CdCl_2$ (5 mg/kg) + EGCG (50 mg/kg)

The dose of $CdCl_2$, vitamin C and EGCG were selected based on available literature.[12-14] $CdCl_2$, Vitamin C and EGCG were dissolved in distilled water for injection and administered intraperitoneally. All the animals were administered with respective assigned treatment once daily for 28 days. The animals in Group I was administered with a drug vehicle *i.e.*, water for injection. During the experiment, body weight changes and behavioral functions such as locomotion, grip strength, and escape latency time (ELT) were monitored at regular time intervals. At the end of the study, the blood samples of the rats were collected for biochemical analysis. Later, the rats were subjected to bilateral orchiectomy; sperm was collected from cauda epididymis for microscopic examination. Then, the animals were sacrificed and organs such as liver, kidney and testis were collected for organ weight analysis.

Body weight analysis: Throughout the study, changes in the body weight of experimental animals were monitored at regular intervals.

Behavioral Analysis: Behavioral functions such as locomotion, grip strength, and ELT were measured using actophotometer, Rotarod and Morris water maze test as per the method described by Parasuraman *et al*.[15] The locomotion and grip strength were measured on pre-study day and, 14 & 28 days of the experiment, and the ELT was measured from day 24 to 28 of the experiment.

Biochemical analysis: At the end of the experiment, few millilitres (mL) of blood samples were collected from all the experimental animals in the micro-centrifuge tube through retro-orbital plexus and the serum was separated by centrifuging at 3000 RPM for 20 minutes. The serum samples were used for biochemical markers estimation such as glucose, Aspartate Aminotransferase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP), urea, creatinine, Total Cholesterol (TC), Triglyceride (TGL) and High-density Lipoprotein (HDL) using Reflotron Plus biochemical analyzer (Roche Diagnostics, Germany) with the help of commercially available Reflotron strips. The cholesterol ratio was calculated mathematically using the formula TC/HDL.

Determination of sperm motility: At the end of the study, sperm samples were collected from cauda epididymis. The cauda epididymis was isolated and placed in a petri dish containing Bigger Whitten and Whittingham (BWW) medium [which consists of (95 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20 mM sodium lactate, 5 mM glucose, 0.25 mM sodium pyruvate and 25 mM $NaHCO_3$, pH 7.4)] at 37°C to allow the sperm to swim in the medium (swim-up technique). An appropriate volume of spermatozoa was transferred and the number of spermatozoa was calculated using the red blood cell count method. From a total of 200 spermatozoa, the active ones were observed using a microscope to calculate sperm motility.[16]

The number of sperm cells in a sample was calculated mathematically using the following formula.

$$\text{No. of sperm cells per cubic mm} = \frac{\text{(No. of sperm counted x dilution)}}{\text{(No. of square mm counted x depth of chamber)}}$$

Determination of sperm count: After completing the determination of sperm motility, the rest of the sperm suspension was quickly transferred to a 60°C water bath for 5–10 min to induce loss activation of sperm, and then carefully added to the cell count plate.

The number of spermatozoa was calculated using the red blood cell counting method.[16]

The number of sperm cells (per cubic mm) in a sample was calculated mathematically using the following formula.

$$= \frac{\text{(No.of sperm counted x dilution)}}{\text{(No.of square mm counted x depth of chamber)}}$$

Organ weight analysis: At the end of the experiment, all the experimental animals were sacrificed under mild ether anesthesia followed by cervical dislocation. The animals were dissected and the gross pathology of organs was observed. The organs such as liver, kidney and testis were harvested. The absolute and relative organ weights were calculated.

Statistical analysis

The results were expressed as mean ± Standard Error of the Mean (SEM). Statistical analysis was carried out using one-way ANOVA followed by Turkey's *post-hoc* test. A value of $p<0.05$ was considered to be significant.

Results and Discussion

Effects of EGCG on Body Weight variations

The effects on the body weights of $CdCl_2$, $CdCl_2$ + vitamin C and $CdCl_2$ + EGCG administered rats were summarized in Figure – 1. The animals administered with $CdCl_2$ showed a significant reduction in body weight from day 14 onwards whereas animals co-administered with vitamin C prevented the reduction of body weight when compared to the control. The animals co-administered with EGCG

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

showed a significant reduction in body weight from day 21 onwards when compared with the control group. Cadmium causes oxidative stress and autophagy thereby reducing body weight.[17]

Effects of EGCG on the Behaviour of the rats

The animals administered with $CdCl_2$ showed a significant decrease in grip strength from day 14 onwards and the animals administered with $CdCl_2$ + EGCG showed a significant decrease in grip strength on day 28 when compared to control (Figure – 2). In the water maze test, the animals administered with $CdCl_2$ showed a significant increase in ELT from day 27 and 28 when compared to the control. Whereas the animals administered with $CdCl_2$ + EGCG showed a significant increase in ELT on day 28 when compared to the control group (Figure 3). In actophotometer, the animals administered with $CdCl_2$ or $CdCl_2$+ vitamin C or $CdCl_2$ + EGCG did not show any significant changes in locomotor activity (Figure 4).

The results showed that the rats administered with $CdCl_2$ showed a decrease in locomotor activities, but the results were not significant different when compared to the control group. Gupta *et al*, reported decrease in motor activity, motor coordination and impairment in forelimb grip strength of rats treated with $CdCl_2$ (5 mg/kg BW; per oral; once daily for 28 days) because of decrease of protein kinase A /DARPP-32 phosphorylation with increased Protein phosphatase 1 alpha (pp1α) phosphorylation which led to alter cAMP response element-binding protein (CREB) levels and affect motor behavior and motor coordination.[18]

Effects of ECGC on Biochemical Parameters

In the biochemical analysis, the animals administered with $CdCl_2$ showed significant increases in the levels of AST, ALT and urea when compare to the control group (Table – 1). Increased levels of AST, ALT and urea with cadmium administration was already well reported in the literature.[19,20] Cadmium also induces hepatotoxicity and renal dysfunction which may be due to the generation of mitochondrial reactive oxygen species (ROS).[20,21] The animals administered with $CdCl_2$ + vitamin C did not show any significant changes in the levels of glucose, AST, ALT, ALP, urea and creatinine, whereas the animals administered with $CdCl_2$ + EGCG showed a significant increase in the levels of urea when compared to the control group. The animals co-administered with vitamin C prevented Cd-induced hepatic and renal enzymes impairments where EGCG prevented only hepatic enzymes impairments when compared with the control group. This may be due to its antioxidant properties.[22] In lipid analysis, a significant decrease in the level of HDL was observed with the animals administered with $CdCl_2$ and $CdCl_2$ + EGCG when compared to the control group (Table – 2). $CdCl_2$ did not show any changes in the levels of TC and TGL. The animals, administered with $CdCl_2$ + EGCG showed a significant increase in the level of cholesterol ratio when compared to control. The decrease in the level of HDL may be due to $CdCl_2$-induced oxidative stress which was inhibited by vitamin C and not by EGCG. In this study, the concentration of ECGC administered may not be adequate to prevent $CdCl_2$-induced oxidative stress. Oyewole *et al.*, reported $CdCl_2$-induced hyperlipidemia and this could be due to the peroxidation of membranes and alteration of the cellular structure by $CdCl_2$.[23]

Effects of EGCG on sperm motility and sperm count

The animals administered with $CdCl_2$ and $CdCl_2$ + EGCG showed a significant decrease in sperm motility (%) when compared with the control group. Whereas the animals administered with $CdCl_2$ + vitamin C did not show any significant changes in sperm motility (%) when compared with the control group (Figure 5).

The sperms were observed under a light microscope and the number of spermatozoa was counted. The animals administered with $CdCl_2$ showed a significant reduction in epididymal sperm count when compared to the control group (Table 3) and this may be due to the production of ROS and peroxidation of the cell membrane.[24] Oxidative stress is also one of the reasons for decreases in sperm count which was observed in the $CdCl_2$ administered group. Oxidative stress significantly increases the production of abnormal sperm and decreases sperm count and transformation, which causes infertility. In high oxygen pressure, spermatozoa increase the production of hydrogen peroxide (H_2O_2) and that induce lipid peroxidation which results in cell death.[25] The testis is extremely sensitive to cadmium toxicity and it causing multiple routes of exposure to induce benign and malignant tumor in animals. Cadmium causes apoptosis (target candidates: p53, c-JNK and Caspase-3), germ cell loss, testicular edema, hemorrhage, necrosis, carcinogenesis (target candidates: antioxidant enzymes, c-fos. C-JNK) and disturbs BTB (target candidates: TGFβ3, p38 MAPK, c-JNK, FAK) in mammalians.[4] Normally, BTB functions will be influenced by cytokine TGF-β3, occludin, zonula occludens (ZO-1), N-cadherin, and claudin-11.[26] The animals administered with $CdCl_2$ + vitamin C did not show any significant changes in epididymal sperm count when compared to the control group.

EGCG abundant in green tea and the extract of green tea exhibited a beneficial effect on $CdCl_2$-induced changes in spermatogenesis and improved rat sperm quality.[24] Sharma and Goyal studied the effect of green tea catechin (7500 µg/kg/animal/day) against $CdCl_2$-induced testicular dysfunctions in mice and reported its testicular protective which is mediated through an antioxidant mechanism.[27] In this study, EGCG did not show any improvement in the rat sperm quality against $CdCl_2$-induced toxicity and also failed to prevent $CdCl_2$-induced behavioural changes.

Effects of EGCG on organ weights

The animals administered with $CdCl_2$ showed a significant reduction in absolute organ weight of epididymal caput/corpus and epididymal cauda when compared to the control group. The animals administered with $CdCl_2$ + vitamin C and $CdCl_2$ + EGCG did not show any significant changes in weight of epididymal caput/corpus and epididymal cauda when compared to the control group (Table – 3). No significant changes were observed in the absolute and relative organ weight of liver, kidney and testis when compared with the control group (data not presented).

In this study, reduction in body weight and reduction in absolute organ weight of epididymal caput/corpus and epididymal cauda were observed with the animals administered with $CdCl_2$. In living cells, protection against oxidative damage encompasses enzymatic (catalase, glutathione peroxidase, glutathione reductase, glutathione-S transferase, superoxide dismutase, and esterases) and non-enzymatic antioxidant (selenium, zinc, and reduced glutathione) systems. Impairment of the enzymatic antioxidant system may attribute to Cd-induced oxidative stress and it reduces relative weight.[28]

Several studies have reported that cadmium toxicity stimulates the production of ROS, induction of oxidative stress in different organs.[6,17]

Table 1: Effects of EGCG on biochemical parameter of the rats

Group	Glucose (mmol/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	6.13 ± 0.25	82.67 ± 2.36	63.67 ± 2.49	86.5 ± 4.69	24.33 ± 1.94	0.25 ± 0.02
$CdCl_2$	6.95 ± 0.47	109.33 ± 6.73**	93.83 ± 4.80**	81.17 ± 5.83	41.5 ± 3.5***	0.27 ± 0.04
$CdCl_2$ + Vitamin C	6.02 ± 0.2	86.67 ± 4.99	64.5 ± 4.62	82.17 ± 9.33	30.5 ± 2.23	0.24 ± 0.01
$CdCl_2$ + EGCG	6.63 ± 0.17	97.5 ± 3.89	83.17 ± 7.07	77.33 ± 6.79	36.33 ± 3.08*	0.27 ± 0.02

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase. Values are expressed as mean ± SEM (n = 6). *$P < N0.05$; **$P < 0.01$ and ***$P < 0.001$ compare with the control group (One-way ANOVA followed by Tukey's *post-hoc* test)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Effects of EGCG on lipid profile of the rats

Group	TC (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	Cholesterol ratio
Control	91.5 ± 3.22	74.83 ± 4.49	23.17 ± 1.25	4.01 ± 0.26
$CdCl_2$	88.5 ± 4.07	70.5 ± 5.33	16.83 ± 1.74*	5.58 ± 0.66
$CdCl_2$ + Vitamin C	84.83 ± 4.07	76.67 ± 5.78	22.67 ± 1.02	3.76 ± 0.19
$CdCl_2$ + EGCG	87.83 ± 5.35	87.5 ± 2.70	15.17 ± 0.98***	5.89 ± 0.49*

TC: Total cholesterol; TGL: Triglyceride; HDL: High-density lipoprotein. Values are expressed as mean ± SEM (n = 6). *$P < 0.05$ and ***$P < 0.001$ compare with the control group (One-way ANOVA followed by Tukey's *post-hoc* test)

Table 3: Effect of EGCG on sperm count and weight of epididymal Caput/ corpus and epididymal Cauda of the rats

Group	Epididymal sperm count (x 10^6/mL)	Absolute organ weight of epididymal caput/corpus (g)	Absolute organ weight of epididymal cauda (g)
Control	17.50 ± 1.54	0.71 ± 0.01	0.48 ± 0.01
$CdCl_2$	8.17 ± 0.48***	0.64 ± 0.03*	0.39 ± 0.02**
$CdCl_2$ + Vitamin C	14.50 ± 1.18	0.70 ± 0.01	0.47 ± 0.01
$CdCl_2$ + EGCG	10.17 ± 0.48**	0.66 ± 0.01	0.42 ± 0.02

Values are expressed as mean ± SEM (n = 6). *$P < 0.05$ and **$P < 0.01$ compare with the control group (One-way ANOVA followed by Tukey's *post-hoc* test)

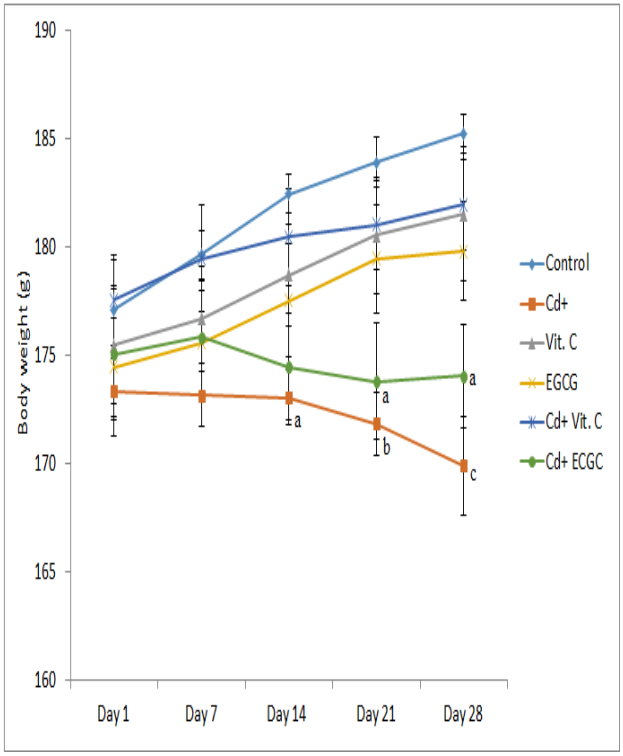

Figure 1: Effects of EGCG on body weight of the rats.
Values are expressed as mean ± SEM (n = 6). [a]P < 0.05; [b]$P < 0.01$ and [c]$P < 0.001$ compare with the control group (One-way ANOVA followed by Tukey's *post-hoc* test)

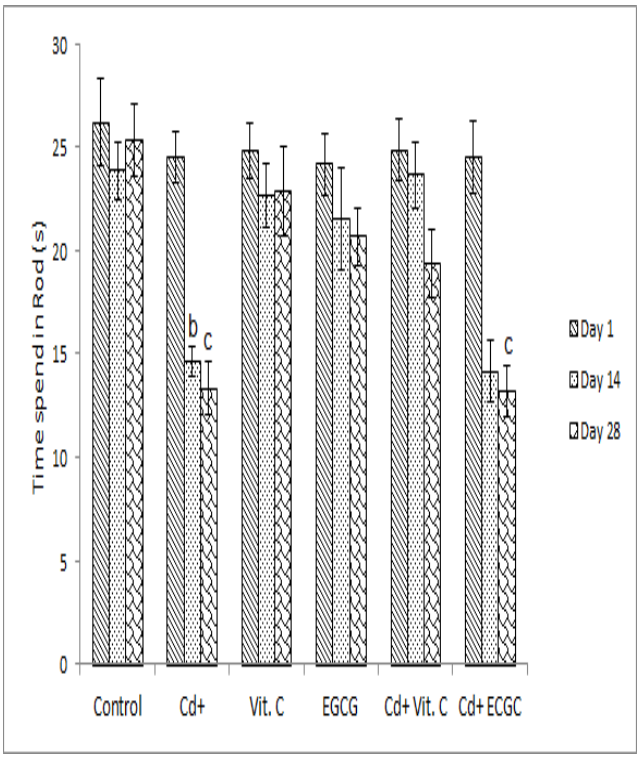

Figure 2: Effects of EGCG on grip strength of the rats.
Values are expressed as mean ± SEM (n = 6). [b]$P<0.01$ and [c]$P<0.001$ compare with the control group (One-way ANOVA followed by Tukey's *post-hoc* test)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

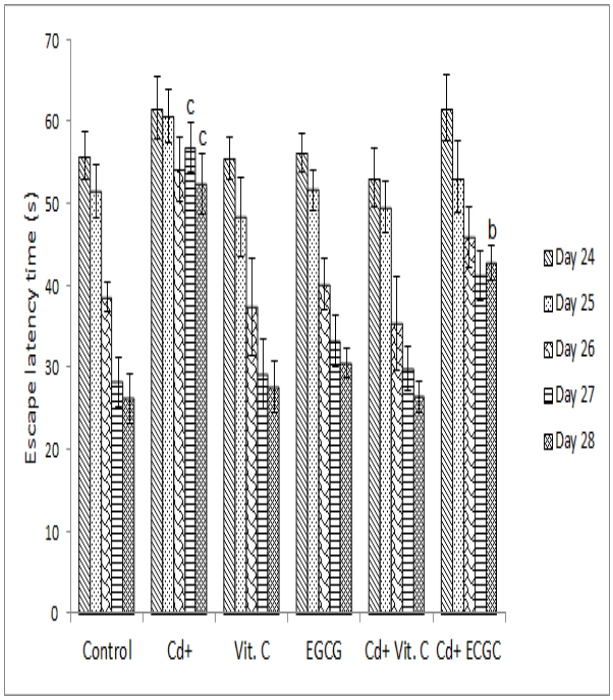

Figure 3: Effect of EGCG on escape latency time of the rats. Values are expressed as mean ± SEM (n = 6). $^{b}P < 0.01$ and $^{c}P < 0.001$ compare with the control group (One-way ANOVA followed by Tukey's *post-hoc* test)

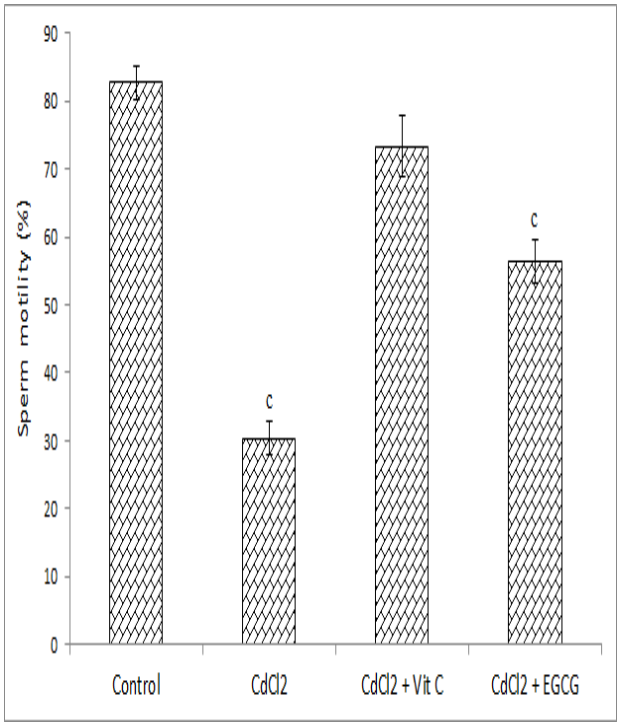

Figure 5: Effects of EGCG on sperm motility**.** Values are expressed as mean ± SEM (n = 6). $^{c}P < 0.001$ compare with the control group (One-way ANOVA followed by Tukey's *post-hoc* test)

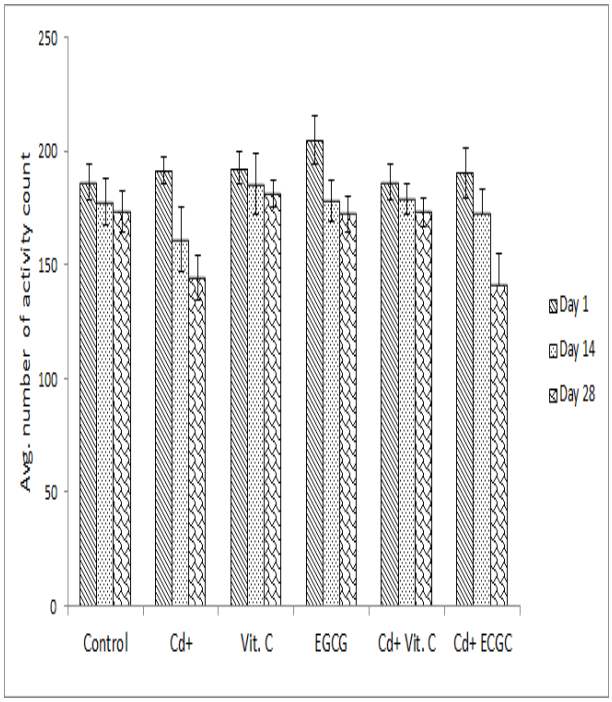

Figure 4: Effect of EGCG on locomotor activity of the rats. Values are expressed as mean ± SEM (n = 6).

Conclusion

EGCG (50 mg/kg) did not show any significant preventive effect against $CdCl_2$-induced behavioural changes of the rats and reduction in the number of sperm cells, whereas vitamin C (200 mg/kg) has an ameliorative effect on $CdCl_2$-induced changes in behavioral functions of the rats and reduction in the number of sperm cells.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Rehman K, Fatima F, Waheed I, Akash MSH. Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem. 2018; 119(1):157-184.
2. Cadmium (Cd) - Chemical properties, Health and Environmental effects [Internet]. [cited 2019 May 15]. Available from: https://www.lenntech.com/periodic/elements/cd.htm
3. Randi AS, Monserrat JM, Rodriguez EM, Romano LA. Histopathological effects of cadmium on the gills of the freshwater fish, *Macropsobrycon uruguayanae Eigenmann* (Pisces, Atherinidae). J Fish Dis. 1996; 19(4):311-322.
4. Su L, Mruk DD, Lee WM, Cheng CY. Drug transporters and blood–testis barrier function. J Endocrinol. 2011; 209(3):337-351.
5. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003; 68(1):167-182.

6. Zhao LL, Ru YF, Liu M, Tang JN, Zheng JF, Wu B, Gu YH, Shi HJ. Reproductive effects of cadmium on sperm function and early embryonic development *in vitro*. PloS one. 2017; 12(11):e0186727.
7. Siu ER, Mruk DD, Porto CS, Cheng CY. Cadmium-induced testicular injury. Toxicol Appl Pharmacol. 2009; 238(3):240-249.
8. Herak-Kramberger CM, Sabolic I, Blanuša M, Smith PJ, Brown D, Breton S. Cadmium inhibits vacuolar H+ ATPase-mediated acidification in the rat epididymis. Biol Reprod. 2000; 63(2):599-606.
9. Chang CJ, Chiu KL, Chen YL, Chang CY. Separation of catechins from green tea using carbon dioxide extraction. Food Chem. 2000; 68(1):109-113.
10. Roychoudhury S, Agarwal A, Virk G, Cho CL. Potential role of green tea catechins in the management of oxidative stress-associated infertility. Reprod Biomed Online. 2017; 34(5):487-498.
11. Singh R, Akhtar N, Haqqi TM. Green tea polyphenol epigallocatechin-3-gallate: inflammation and arthritis. [corrected]. Life Sci. 2010; 86(25-26):907-918.
12. Oladele JO, Oyewole OI, Bello OK, Oladele OT. Hepatoprotective effect of aqueous extract of *Telfairia occidentalis* on cadmium chloride-induced oxidative stress and hepatotoxicity in rats. J Drug Design Med Chem. 2017; 3(3):32-36.
13. Giordano V, Albuquerque RP, Amaral NP, Chame CC, Souza FD, Apfel MÍ. Supplementary vitamin C does not accelerate bone healing in a rat tibia fracture model. Acta ortopedica brasileira. 2012; 20(1):10-12.
14. Nna VU, Ujah GA, Mohamed M, Etim KB, Igba BO, Augustine ER, Osim EE. Cadmium chloride-induced testicular toxicity in male wistar rats; prophylactic effect of quercetin, and assessment of testicular recovery following cadmium chloride withdrawal. Biomed Pharmacother. 2017; 94:109-123.
15. Parasuraman S, Hoong SS, Christapher PV, Zou LN, De Wei DL, Loshini S, Ching TH, Leong CH.. Effect of ethanolic extract of leaves of *Solanum trilobatum* on scopolamine-induced memory impairment in Sprague Dawley rats. J Pharm Negative Results 2019; 10:41-46.
16. He L, Li P, Yu LH, Li L, Zhang Y, Guo Y, Long M, He JB, Yang SH. Protective effects of proanthocyanidins against cadmium-induced testicular injury through the modification of Nrf2-Keap1 signal path in rats. Environ Toxicol Pharmacol. 2018; 57:1-8.
17. Wang J, Zhu H, Wang K, Yang Z, Liu Z. Protective effect of quercetin on rat testes against cadmium toxicity by alleviating oxidative stress and autophagy. Environ Sci Pollut Res. 2020; 27(20):25278-25286.
18. Gupta R, Shukla RK, Pandey A, Sharma T, Dhuriya YK, Srivastava P, Singh MP, Siddiqi MI, Pant AB, Khanna VK. Involvement of PKA/DARPP-32/PP1α and β-arrestin/akt/GSK-3β signaling in cadmium-induced DA-D2 receptor-mediated motor dysfunctions: protective role of quercetin. Sci Rep. 2018; 8(1):1-8.
19. Santos FW, Zeni G, Rocha JB, Weis SN, Fachinetto JM, Favero AM, Nogueira CW. Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. Chem Biol Interact. 2005; 151(3):159-165.
20. Renugadevi J and Prabu SM. Naringenin protects against cadmium-induced oxidative renal dysfunction in rats. Toxicology. 2009; 256(1-2):128-134.
21. Pi H, Xu S, Reiter RJ, Guo P, Zhang L, Li Y, Li M, Cao Z, Tian L, Xie J, Zhang R. SIRT3-SOD2-mROS-dependent autophagy in cadmium-induced hepatotoxicity and salvage by melatonin. Autophagy. 2015; 11(7):1037-1051.
22. Avadhani KS, Manikkath J, Tiwari M, Chandrasekhar M, Godavarthi A, Vidya SM, Hariharapura RC, Kalthur G, Udupa N, Mutalik S. Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation induced skin damage. Drug Deliv. 2017; 24(1):61-74.
23. Oyewole OI, Shoremi MO, Oladele JO. Modulatory effects of *Ricinus communis* leaf extract on cadmium chloride-induced hyperlipidemia and pancytopenia in rats. Am J Biomed Res. 2016; 4(2):38-41.
24. Mahmoudi R, Azizi A, Abedini S, Hemayatkhah Jahromi V, Abidi H, Jafari Barmak M. Green tea improves rat sperm quality and reduced cadmium chloride damage effect in spermatogenesis cycle. J Med Life. 2018; 11(4):371-380.
25. Sanocka D and Kurpisz M. Reactive oxygen species and sperm cells. Reprod Biol Endocrinol. 2004; 2:12.
26. Siu ER, Wong EW, Mruk DD, Sze KL, Porto CS, Cheng CY. An occludin-focal adhesion kinase protein complex at the blood-testis barrier: a study using the cadmium model. Endocrinol. 2009; 150(7):3336-3344.
27. Sharma P and Goyal PK. Ameliorative effect of green tea catechin against cadmium chloride-induced testicular toxicity in mice. J Environ Pathol Toxicol Oncol. 2015; 34(4):335-352.
28. Elmallah MIY, Elkhadragy MF, Al-Olayan EM, Abdel Moneim AE. Protective effect of *fragaria ananassa* crude extract on cadmium-induced lipid peroxidation, antioxidant enzymes suppression, and apoptosis in rat testes. Int J Mol Sci. 2017; 18(5):957.