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Effect of epigallocatechin gallate on aluminum chloride-induced changes in behavior, biochemical parameters, and spermatogenesis of Sprague-Dawley rats

Subramani Parasuraman*, Brenda Ngu Yen Qin, Lam Chew Hui and James Yu Kar Beng

Abstract

Background: Epigallocatechin gallate (EGCG) acts as an antioxidant by preventing oxidative stress. The effect of EGCG on aluminum-induced testicular injury is not clear. Hence, the present study is planned to investigate the effect of EGCG on aluminum chloride ($AlCl_3$)-induced changes in behavior, biochemical parameters, and spermatogenesis in male *Sprague-Dawley* rats. The rats were divided into six groups with six animals each. All the animals were administered with respective assigned treatment once daily for 28 days. The animals in groups I to VI were administered with drug vehicle, $AlCl_3$, vitamin C, EGCG, vitamin C, and EGCG, respectively. The animals in groups V and VI were additionally challenged with $AlCl_3$ (10 mg/kg) immediately after vitamin C and EGCG administration, respectively. Changes in behavior were measured on day 1, 14 and 28. At the end of the study, the blood sample was collected from all the animals, and the serum was separated and used for biochemical analysis. Later, the rats were subjected to bilateral orchiectomy; sperm was collected from the cauda epididymis for microscopic examination. Then, the animals were sacrificed, and the organs such as the brain, lungs, heart, liver, kidney, spleen, and testis were collected for organ weight analysis.

Results: The animal administered with $AlCl_3$ showed a reduction in locomotor activity, grip strength, and escape latency time whereas vitamin C prevented the effect of $AlCl_3$. But, EGCG did not show any significant changes in $AlCl_3$-induced behavioral and biochemical changes. At the end of the study, vitamin C prevented $AlCl_3$-induced behavioral and biochemical changes. The group of animals administered with $AlCl_3$ showed a reduction in the number of spermatozoa whereas $AlCl_3$ + vitamin C and $AlCl_3$ + EGCG did not show any significant changes in the number of spermatozoa when compared to the control group.

Conclusion: EGCG prevented $AlCl_3$-induced reduction in epididymal sperm count of male rats and did not show any significant effect on $AlCl_3$-induced changes in behavior and biochemical parameters, whereas vitamin C had an ameliorative effect on $AlCl_3$-induced changes in behavior, biochemical parameter, and spermatogenesis.

Keywords: Antioxidant, Catechin, Escape latency time, Grip strength, Locomotor activity, Muscle coordination, Orchiectomy

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1 Background

Aluminum (Al) is the third most common element that can be found in the earth's crust and also found in soil, water, and air [1]. The physical and chemical properties of this element make it supreme for a variety of uses in food, drugs, consumer products, and water treatment processes. Al is a compound that has been incorporated into different kinds of products such as antacids, phosphate binders, buffered aspirins, vaccines, and allergen injections as well as into consumer products such as antiperspirants, first-aid antibiotic, antiseptics, and food additives [2]. In recent years, the potential adverse health effects of Al that are present in drinking water had raised concerns among the community.

Exposure to Al in drinking water is associated with health risks which can be acute and chronic toxicity. For acute toxicity, there is little indication that Al is acutely toxic by oral exposure, despite its widespread occurrence in foods, drinking water, and many antacid preparations [3]. There are no reported cases of acute Al poisoning of healthy individuals exposed to normal levels of Al, which is below 0.2 mg/L [4]. Severe diseases of the nervous system such as Parkinson's dementia, amyotrophic lateral sclerosis, and Alzheimer's disease are associated with chronic Al toxicity [5]. High consumption of Al-containing products will increase the concentration of this metallic element in the consumers' organs and damage their various tissues including the testicular tissues [6, 7]. Krasovskiĭ et al. have confirmed the gonadal toxicity of lead and aluminum chloride ($AlCl_3$) in guinea pigs and rats [8]. Al decreases sperm count, daily sperm production and sperm motility and low concentrations of Al in testes (3.35 μg/g) are sufficient to impair spermatogenesis and sperm quality [9]. $AlCl_3$ also decreases the body weight, weights of testis or epididymis, number of normal sperm cells, sperm concentration and motility in experimental animals [10]. A significant decrease in fertility in female and male rats and the necrosis of spermatocytes/spermatids were found due to the accumulation of Al in the testis [11, 12]. Accumulation of Al will also cause male reproductive toxicity, and this may be mediated through various mechanisms inducing oxidative stress, interfering with spermatogenesis and steroidogenesis, impairing cell signaling, disrupting the blood-testis barrier, and affecting the endocrine system [6].

In recent years, natural antioxidants are gaining interest in the management of diseases related to oxidative stress. Epigallocatechin gallate (EGCG) is one of the natural antioxidants; an active component of green tea can serve as an antioxidant and therapeutic agent [13]. EGCG is the ester of epigallocatechin and gallic acid and/or the major polyphenolic constituent, abundant in dried fresh leaves of the plant *Camellia sinensis* (family Theaceae) [12]. *C. sinensis*/tea plant/green tea also contains other catechins such as (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (+)-catechin [10]. EGCG and other catechins are the phytoconstituents of green tea and responsible for its potential health benefits [11]. EGCG is claimed to have a powerful antioxidant effect and can decrease stress and inflammation. Highly reactive particles such as free radicals can cause oxidative damage to human cells, and overproduction of the radicals will cause oxidative stress. In this case, EGCG acts as an antioxidant agent by preventing oxidative stress and also suppresses the activity of pro-inflammatory chemicals [14]. Different kinds of chronic illnesses such as cancer, diabetes, and heart disease are related to stress and inflammation, and EGCG is useful in preventing diseases by acting as an antioxidant agent [15, 16]. The effect of EGCG on Al-induced testicular injury is not clear. Hence, the present study is planned to investigate the effect of EGCG on $AlCl_3$-induced changes in behavior, biochemical parameters, and spermatogenesis in male *Sprague-Dawley* (SD) rats.

2 Methods

2.1 Chemicals

Calcium chloride ($CaCl_2$), monopotassium phosphate (KH_2PO_4), sodium lactate, and vitamin C were purchased from R&M Marketing, Essex, UK. $AlCl_3$ and glucose were purchased from Friendemann Schmidt Chemical, USA. Analytical-grade sodium chloride (NaCl), potassium chloride (KCl), magnesium sulfate ($MgSO_4$), and sodium bicarbonate ($NaHCO_3$) were purchased from Bendosen, Malaysia. Sodium pyruvate was purchased from Sigma Chemical, USA. EGCg green tea extract—400-mg capsules—were purchased from Now Foods, USA. Each capsule of EGGg contains 200 mg of EGCG.

2.2 Animals

Healthy, adult, male SD rats with a weight of 180 ± 20 g were used for the study. The rats were housed and maintained in large, spacious polyacrylic cages at ambient room temperature with a 12-h light/12-h dark cycle. The animals were fed with water and normal rat pellet diet *ad libitum*. The study was approved by the University Human and Animal Ethics Committee (AUAEC/FOP/2019/09), and the study was conducted according to the Animal Research Review Panel guidelines.

2.3 Experimental design

Healthy, adult male SD rats were used for the study. The animals were divided into six groups with six animals each as follows:

Group I: control

Group II: $AlCl_3$ (10 mg/kg)
Group III: Vitamin C (200 mg/kg)
Group IV: EGCG (50 mg/kg)
Group V: $AlCl_3$ (10 mg/kg) + vitamin C (200 mg/kg)
Group VI: $AlCl_3$ (10 mg/kg) + EGCG (50 mg/kg)

The doses of vitamin C and EGCG were selected based on the available literature [17, 18]. $AlCl_3$ (10 mg/kg) was used to induce oxidative stress [19]. Vitamin C, EGCG, and $AlCl_3$ 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 groups I to VI were administered with drug vehicle, $AlCl_3$, vitamin C, EGCG, vitamin C, and EGCG, respectively. The animals in groups V and VI were additionally challenged with $AlCl_3$ (10 mg/kg) immediately after vitamin C and EGCG treatment. The changes in body weight and behavior were measured at regular intervals [20]. At the end of the study, the blood sample was collected from all the animals, and the serum was separated. The serum samples were used for biochemical analysis. Later, the rats were subjected to bilateral orchiectomy; sperm was collected from the cauda epididymis for microscopic examination [21, 22]. Then, the animals were sacrificed, and the organs such as the brain, lungs, heart, liver, kidney, spleen, and testis were collected for organ weight analysis.

2.4 Body weight analysis

The body weight (BW) of each rat in each group was recorded initially and at 3 days intervals. The change in BW was calculated.

2.5 Behavior and muscular activities

At regular intervals, behavioral (locomotor activity), muscular coordination (rotarod and wire grip test), and memory function (Morris water maze test) were evaluated.

2.5.1 Locomotor activity

The activity of the rats was recorded in a rat activity cage (actophotometer) which is constructed with an acrylic cage and 8 beams of infrared light along both the x and y-axes. The activity of each rat was monitored at room temperature over 10 min.

2.5.2 Muscle coordination (rotarod) test

The rat was placed on a rotarod with a speed of 20 RPM. The time between rat maintains on the rotarod and when it fall was recorded. The procedure was repeated for each rat.

2.5.3 Hanging wire grip test

The string is made up of metallic wire and suspended in mid-air about 30 cm height from the ground. The rat was placed on the center of the wire, and the time taken to fall, i.e., "fall on time," was noted.

2.5.4 Morris water maze test

The water navigation test is employed as a method to test spatial learning and memory parameters to evaluate spatial learning and memory functions. The training was taken place for 3 consecutive days, with four consecutive trials/day for each experimental rat at the inter-trial interval of 30 min. In the pre-study training sessions, if the animals failed to escape on the platform within 180 s, they will be excluded from the study.

The Morris water maze consists of a round pool, filled with tap water, which is close to 23–26 °C, at a depth of 0.3–0.4 m. The pool was divided into four hypothetical quadrants, with an escape platform placed 1 cm below the water surface at the center. Four different starting points for rats were placed around the perimeter of the pool. The test was performed from day 24 onwards at day intervals. Escape latency time (ELT) to locate the hidden platform in the water maze was noted as an index of learning.

2.6 Quality evaluation of semen

2.6.1 Determination of sperm motility

At the end of the study, sperm samples were collected from the cauda epididymis. The cauda epididymis was isolated and placed in a petri dish contacting Biggers, Whitten, and Whittingham (BWW) [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] medium to allow the sperm to swim (swim-up technique). An appropriate volume of spermatozoa was transferred to a preheated cell count plate. From a total of 200 spermatozoa, the active ones were observed using a microscope to calculate sperm motility [23].

2.6.2 Determination of sperm count

After completing the determination of sperm motility, the rest of the sperm suspension was quickly transferred to 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 [24].

2.7 Biochemical analysis

At the end of the experiment, a few milliliters (mL) of the blood sample was collected in the micro-centrifuge tube through retro-orbital plexus and the serum was

separated by centrifuging at 3000 RPM for 20 min. The serum samples were used for biochemical marker estimation such as glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine and, urea, and lipid parameters such as total cholesterol (TC), triglyceride (TGL), and high-density lipoprotein (HDL) using the Reflotron Plus biochemical analyzer (Roche Diagnostics, Germany) with the help of commercially available Reflotron strips. Very low-density lipoprotein (VLDL) and cholesterol ratio were calculated mathematically [20].

2.8 Gross pathology and 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 was observed. The organs such as the brain, lungs, heart, liver, kidney, spleen, and testis were harvested; absolute organ weights were measured, and relative organ weights were calculated.

2.9 Statistical analysis

Data were represented 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 $P < 0.05$ shall be considered to be significant.

3 Results

3.1 Effects of EGCG on body weight variations

The effects on the body weights of $AlCl_3$-, vitamin C-, EGCG-, $AlCl_3$ + vitamin C-, and $AlCl_3$ + EGCG-administered male rats were summarized in Fig. 1. At the end of the study, $AlCl_3$- and $AlCl_3$ + EGCG-administered animals showed a significant reduction in body weight when compared to the control group. The animals, administered with vitamin C and EGCG alone, did not show any significant changes in regular body weight gain when compared to the control group.

3.2 Effects of EGCG on the behavior of the rats

The animals administered with $AlCl_3$ and $AlCl_3$ + EGCG showed significant decreases in locomotor activity (Fig. 2), grip strength (Fig. 3), and ELT (Fig. 4) when compared to the control group, whereas the animals administered with vitamin C and EGCG alone did not show any significant changes when compared to the control group. In the rotarod experiment, the animals administered with $AlCl_3$ + EGCG only showed significant decreases in muscular strength (Fig. 5), whereas the other groups did not show any significant changes in the "fall on time" when compared to the control group.

3.3 Effects of ECGC on biochemical parameters

In the biochemical analysis, the animals administered with $AlCl_3$ and $AlCl_3$ + EGCG showed significant increases in the levels of glucose, AST, ALT, urea, and creatinine when compared to the control group (Table 1). The animals administered with vitamin C and EGCG did not show any significant changes in the levels of glucose, AST, ALT, ALP, urea, and creatinine when compared to the control group. In lipid analysis (TC, TGL, HDL, and VLDL), no significant changes were observed in the animals administered with $AlCl_3$, vitamin C, EGCG, $AlCl_3$ + vitamin C, and $AlCl_3$ + EGCG when compared to the control group (Table 2).

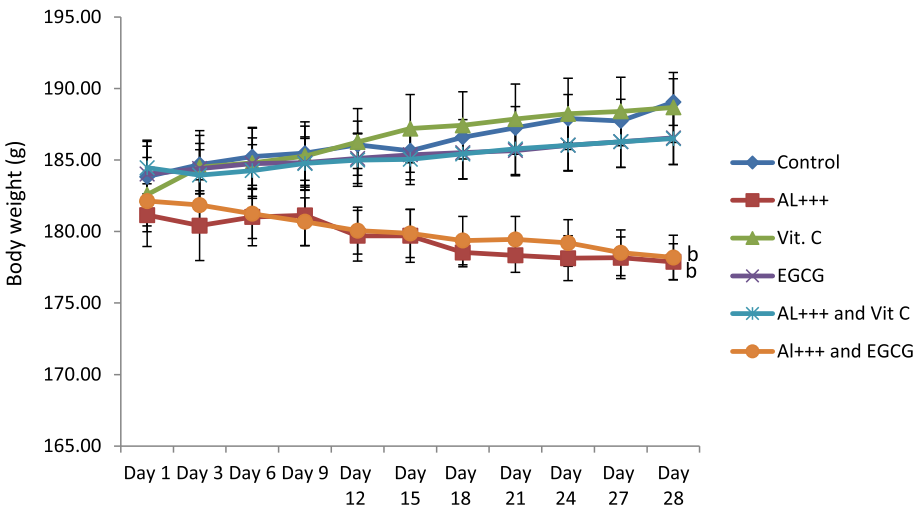

Fig. 1 Effect of aluminum, vitamin C, EGCG, aluminum + vitamin C, and aluminum + EGCG on regular body weight growth of animals. Values are expressed as mean ± SEM ($n = 6$). $^{b}P < 0.01$ compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

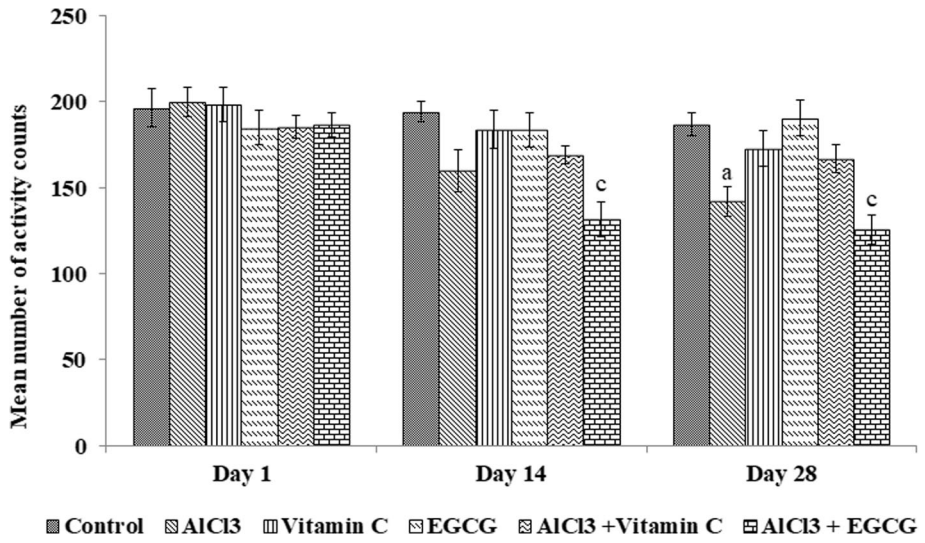

Fig. 2 Effect of EGCG on locomotor activity of the rats. Values are expressed as mean ± SEM ($n = 6$). $^{a}P < 0.05$ and $^{c}P < 0.001$ compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

3.4 Effects of EGCG on organ weight of rats

No changes in gross pathology between the treated and control groups were observed. The animals administered with $AlCl_3$, vitamin C, EGCG, $AlCl_3$ + vitamin C, and $AlCl_3$ + EGCG did not show any significant changes in absolute and relative organ weights of the brain, lung, heart, liver, kidney, spleen, and testis when compared to the control group.

3.5 Effects of EGCG on quality of semen

The sperms were observed under a light microscope. Reduction in the number of viable sperm cells was observed in the animals administered with $AlCl_3$ whereas the other groups did not show any significant changes when compared to the control group (Fig. 6). The animals administered with $AlCl_3$ showed a significant reduction in the number of sperm cells compared to the

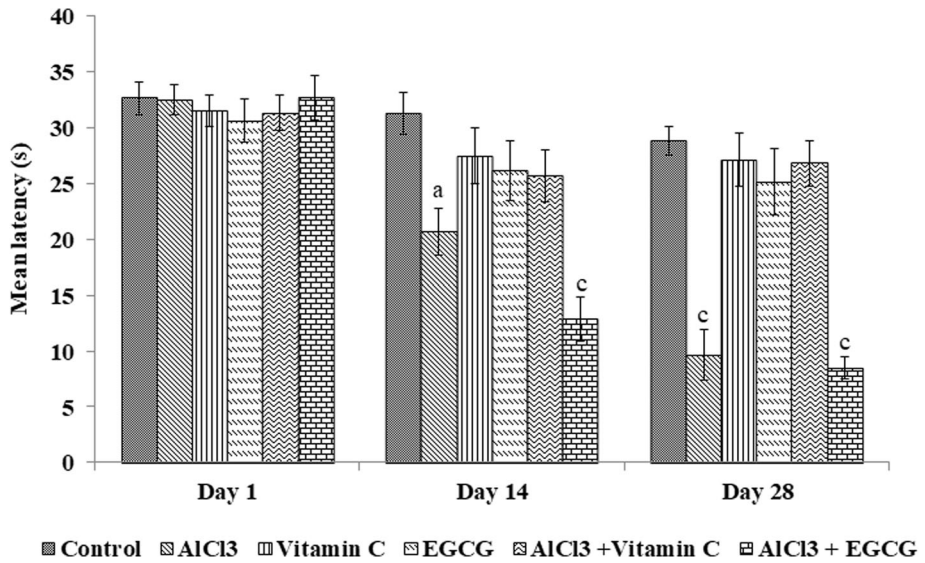

Fig. 3 Effect of EGCG on wire grip strength of the rats. Values are expressed as mean ± SEM ($n = 6$). $^{a}P < 0.05$ and $^{c}P < 0.001$ compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

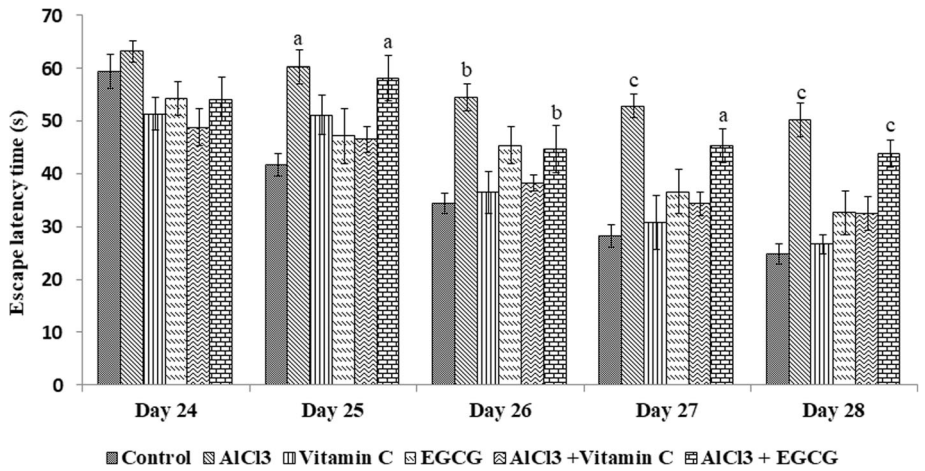

Fig. 4 Effect of EGCG on escape latency time of the rats. Values are expressed as mean ± SEM ($n = 6$). $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$ compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

control group. The animals administered with $AlCl_3$ + vitamin C or EGCG showed a quantitative reduction in sperm count, but the results were not significant (Table 3). The weight of the epididymis caput/corpus and the epididymis cauda did not show any significant variation compared to the control group.

4 Discussion

In recent years, natural antioxidants are gaining interest in the management of diseases related to oxidative stress. EGCG is a natural antioxidant; an active component of green tea can serve as an antioxidant and therapeutic agent [13]. There were a number of proven effects of EGCG on human pathological and physiological processes, and its mechanisms in various systems and diseases are reported elsewhere [25, 26]. Male reproductive toxicity occurs due to the accumulation of Al, and these are due to various mechanisms such as inducing oxidative stress, interfering with spermatogenesis and steroidogenesis, impairing cell signaling, disrupting the blood-testis barrier, and affecting the endocrine system [6].

In the present study, the effect of EGCG on $AlCl_3$-induced changes in behavior, biochemical parameters, and spermatogenesis was studied. The animals administered with $AlCl_3$ showed a decrease in body weight, and the same was reported elsewhere [27, 28].

The animals administered with $AlCl_3$ showed a significant reduction in locomotor activity and grip strength whereas the animals co-administered with vitamin C prevented behavior alteration induced by $AlCl_3$.

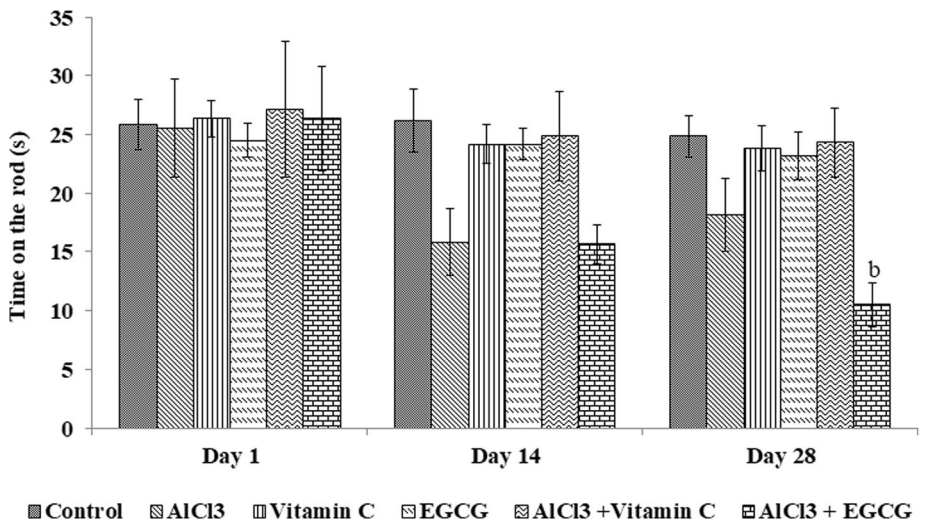

Fig. 5 Effect of EGCG on muscular coordination of the rats. Values are expressed as mean ± SEM ($n = 6$). $^{b}P < 0.05$ compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

Table 1 Effect of EGCG on biochemical parameters of the rats

Group	Glucose (mmol/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	5.8 ± 0.28	83.6 ± 2.70	47.1 ± 3.91	104.33 ± 6.01	20.33 ± 1.49	0.29 ± 0.01
$AlCl_3$	8.05 ± 0.50*	113.33 ± 9.00*	76.1 ± 8.40*	125.83 ± 9.76	38.0 ± 2.98***	0.437 ± 0.04**
Vitamin C	5.93 ± 0.29	78.17 ± 5.59	51.17 ± 5.71	103.67 ± 6.18	21.6 ± 1.33	0.26 ± 0.02
EGCG	5.83 ± 0.23	87.83 ± 3.83	48.33 ± 3.11	110.33 ± 5.89	21.33 ± 1.76	0.32 ± 0.01
$AlCl_3$ + vitamin C	6.78 ± 0.69	80.83 ± 4.44	46.83 ± 4.70	106.33 ± 3.48	22.83 ± 2.40	0.327 ± 0.02
$AlCl_3$ + EGCG	8.55 ± 0.72**	122.67 ± 11.38**	82.67 ± 6.17**	127.17 ± 7.33	40.33 ± 2.51***	0.483 ± 0.01***

Values are expressed as mean ± SEM (n = 6). *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

Nampoothiri et al. reported the reduction of locomotor activity with $AlCl_3$ administration, indicating the central nervous system depressant effect of chronic Al exposure [29]. The impaired spatial memory and behavioral function were observed in $AlCl_3$-administered animals which indicate the development of neurotoxicity [30]. Lal et al. also demonstrated decreased locomotor activity, increased brain lipid peroxidation, and decreased brain Mg-ATPase and Nak-ATPase in rats exposed to aluminum (500 mg Al/L in drinking water) daily for 180 days [31]. In the rotarod experiment, $AlCl_3$ co-administered with EGCG showed a significant reduction in muscular strength when compared to the control group, and this may be due to high Al levels in the brain [32]. In this study, EGCG did not show any neuroprotective effect against $AlCl_3$-induced neurotoxicity. But the neuroprotective effect of EGCG was demonstrated elsewhere against quinolinic acid, D-galactose, and hypoxia-ischemia-induced neurotoxicity [33–35].

The animals treated with $AlCl_3$ increased ELT in the Morris water maze test, and this test is more specific for hippocampal function. The rats administered with $AlCl_3$ took a longer time to reach the visible platform in the Morris water maze test compared to the control group from day 25 onwards, and this indicates memory deficits and reduced spatial memory [28]. EGCG co-administration failed to reverse the $AlCl_3$-induced memory deficits.

In the biochemical analysis, the animals administered with $AlCl_3$ showed significant increases in the levels of glucose, AST, ALT, urea, and creatinine compare to the control group, and EEST failed to prevent the $AlCl_3$ effects on biochemical parameters. Biomarkers such as AST, ALT, and ALP were used to check liver functions, and urea and creatinine were used to check the renal functions. The elevated levels of these biomarkers indicating that the animals administered with $AlCl_3$ have the risk of liver and kidney damage. $AlCl_3$ is a known hepatotoxicant, and it may increase the levels of liver enzymes by reducing the electron transport chain complex I–V activities and adenosine triphosphate levels and disturb the mitochondrial DNA transcript [36]. The nephrotoxicity induced by $AlCl_3$ is may be due to the accumulation of Al in the kidney, eventually resulting in renal failure [37]. The animals administered with vitamin C, EGCG, and $AlCl_3$ + vitamin C did not show any abnormalities in biochemical parameters. In both in vitro and in vivo experiments, EGCG showed significant hepatoprotective effect bromobenzene and alcohol-induced hepatotoxicity. Kagaya et al. studied the effect of EGCG on bromobenzene-induced hepatotoxicity in rat hepatocyte cells and reported hepatoprotective 0.02 mM EGCG + 0.02 mM zinc. In this study, EGCG alone did not show any hepatoprotective activity at 0.02 mM against bromobenzene-induced hepatotoxicity [38]. Zhang et al. studied the effect of EGCG on alcohol-induced hepatotoxicity in HepG2 cell line and male Kunming mice. In both experiments, EGCG alleviated the changes induced by alcohol in a dose-dependent manner, and this may be mediated via its antioxidant activity [39]. Bao and Peng demonstrated the nephroprotective effect of EGCG in a chronic kidney disease model (decreased serum creatinine levels) [40]. But, in this study, EGCG did not show any significant hepatoprotective and nephroprotective

Table 2 Effect of EGCG on the lipid profile of the rats

Group	TC (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	Cholesterol ratio
Control	104.83 ± 4.15	84.6 ± 2.71	18.5 ± 1.52	16.93 ± 0.54	5.88 ± 0.58
Aluminum	105.83 ± 4.00	78.67 ± 4.78	16.83 ± 0.54	15.73 ± 0.95	6.32 ± 0.31
Vit. C	108.1 ± 3.07	84.67 ± 4.73	19.67 ± 0.802	16.93 ± 0.95	5.54 ± 0.25
EGCG	103.6 ± 4.84	80.83 ± 2.926	19.167 ± 1.56	16.167 ± 0.59	5.54 ± 0.419
Aluminum + vit. C	102.8 ± 2.71	83.33 ± 3.77	17.83 ± 1.27	16.67 ± 0.75	5.861 ± 0.29
Aluminum + EGCG	104.83 ± 3.95	98.83 ± 3.95	15.67 ± 0.76	19.7 ± 0.79	6.74 ± 0.33

Values are expressed as mean ± SEM (n = 6)

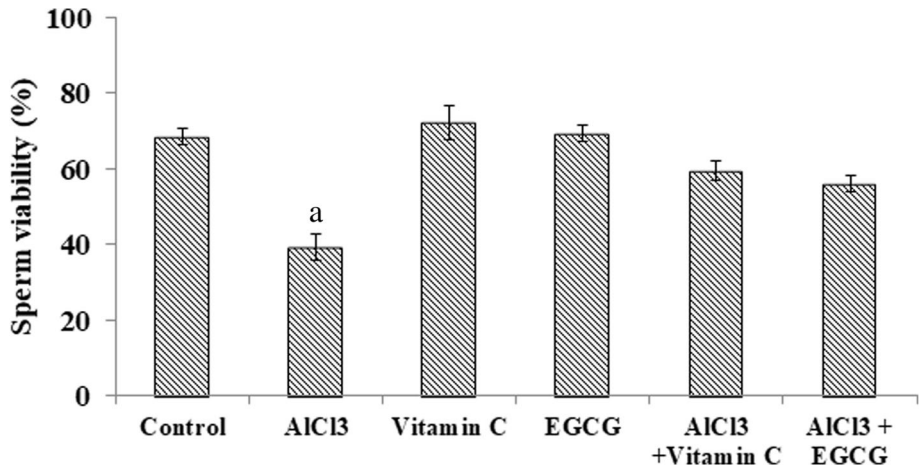

Fig. 6 Effect of EGCG on percentage sperm viability. Values are expressed as mean ± SEM ($n = 6$). $^{a}P < 0.05$ compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

activities against $AlCl_3$-induced Hepato- and nephrotoxicity. In lipid analysis, no significant changes in the levels of TC, TGL, and HDL were observed in all experimental groups when compared to the control group. Yang et al. studied the effect of EGCG and ECCG co-administered with caffeine in obese rats and reported both low dose of ECCG (40 mg/kg) and caffeine (20 mg/kg) has a mild anti-obesity effect and a high dose of EGCG (160 mg/kg) and combination (ECCG [40 mg/kg] + caffeine [20 mg/kg]) exhibits superior curative effect [41].

Analysis of organ weight was used in toxicology studies for the identification of potentially harmful effects of chemicals. In this experiment, no significant changes in absolute and relative organ weight were observed in all experimental groups when compared to the control group.

The reduction in sperm viability is observed in $AlCl_3$-administered animals. Hadi and Deaibil also reported a decrease in the percentage of sperm viability in $AlCl_3$-administered animals, and this may be due to mitochondrial dysfunction [42]. In epididymal sperm count analysis, only those administered with $AlCl_3$ showed a significant reduction in sperm count. Cheraghi et al. also reported the effect of $AlCl_3$ on sperm count, and in his study, the $AlCl_3$-administered group showed > 50% reduction in the number of sperm cells compared to the control group [18]. The quality of the sperm including motility and viability is also affected by Al *via* the Nrf-2/HO-1 signaling pathway [18, 43]. In this study, the animals administered with $AlCl_3$ + vitamin C and $AlCl_3$ + EGCG showed a qualitative reduction in the number of sperm cells when compared to the control group, but the results were not significant.

5 Conclusion

EGCG did not show any significant effect on $AlCl_3$-induced changes in behavior (locomotor activity, grip strength, escape latency time) and biochemical parameters, but it prevented $AlCl_3$-induced reduction in epididymal sperm count of male rats. However, vitamin C had shown the ameliorative effect on $AlCl_3$-induced changes in behavior, biochemical parameters, and spermatogenesis of rats.

Table 3 Effect of EGCG on sperm count and weight of the epididymis caput and epididymis cauda in SD rats

	Epididymal sperm count (× 10^6/mL)	Weight of the epididymis caput/corpus (g)	Weight of the epididymis cauda (g)
Control	17.17 ± 1.58	0. 73 ± 0.01	0.48 ± 0.02
$AlCl_3$	10.33 ± 0.95*	0.65 ± 0.04	0.39 ± 0.02
Vitamin C	16.67 ± 1.26	0.70 ± 0.02	0.46 ± 0.02
EGCG	16.50 ± 1.52	0.72 ± 0.01	0.47 ± 0.02
$AlCl_3$ + vitamin C	13.67 ± 1.38	0.71 ± 0.01	0.46 ± 0.03
$AlCl_3$ + EGCG	11.67 ± 1.12	0.67 ± 0.01	0.47 ± 0.01

Values are expressed as mean ± SEM ($n = 6$). *$P < 0.05$ compared with control (one-way ANOVA followed by Tukey's *post-hoc* test)

6 Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-020-00079-3.

Additional file 1. The ARRIVE Guidelines Checklist. (PDF 1066 kb)

Abbreviations

AChE: Acetylcholinesterase; Al: Aluminum; $AlCl_3$: Aluminum chloride; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BW: Body weight; $CaCl_2$: Calcium chloride; DNA: Deoxyribonucleic acid; EC: (−)-Epicatechin; ECG: (−)-Epicatechin-3-gallate; EGC: (−)-Epigallocatechin; EGCG: Epigallocatechin gallate; ELT: Escape latency time; HDL: High-density lipoprotein; KCl: Potassium chloride; KH_2PO_4: Monopotassium phosphate; $MgSO_4$: Magnesium sulfate; NaCl: Sodium chloride; $NaHCO_3$: Sodium bicarbonate; RPM: Revolutions per minute; *SD* rats: *Sprague-Dawley* rats; SEM: Standard error of the mean; TC: Total cholesterol; TGL: Triglyceride; TNF-alpha: Tumor necrosis factor-alpha; VLDL: Very low-density lipoprotein

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Authors' contributions

Subramani Parasuraman drafted the hypothesis for the current work and contributed to the protocol development, result analysis, manuscript preparation, and review. Brenda Ngu Yen Qin, Lam Chew Hui, and James Yu Kar Beng are the undergraduate students and they carried out the laboratory work, involved in manuscript and presented the thesis to the faculty. The authors read and approved the final manuscript.

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Ethics approval and consent to participate

The study was approved by the AIMST University Human and Animal Ethics Committee (AUAEC/FOP/2019/09), and the study was conducted according to the Animal Research Review Panel guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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