ORIGINAL ARTICLE

Effect of hesperidin in the prevention of aluminum chloride-induced testicular dysfunction in rats

Subramani Parasuraman[1,2], Loh Yee Huey[1], Yap Shen Jee[1]

[1]Unit of Pharmacology, Toxicology and Basic Health Sciences, Faculty of Pharmacy, AIMST University, Bedong, Kedah, Malaysia, [2]Center for Global Health Research, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India

J. Adv. Pharm. Technol. Res.

ABSTRACT

Hesperidin is a citrus bioflavonoid and has numerous pharmacological actions. Hesperidin's effect on testicular dysfunction has not been well researched. Hence, the present study is aims to investigate the effect of hesperidin on aluminium chloride ($AlCl_3$)-induced testicular dysfunction in rats. Both vitamin C (200 mg/kg) and hesperidin (50, 100, and 200 mg/kg) were administered orally for 21 days. At the end of the study, the blood samples were obtained from all animals for investigation of biochemical and hematological parameters. Then, bilateral orchiectomy was carried out to remove testicles from the animals, and sperm was collected and examined under a microscope. Finally, organs such as the liver, kidney, and testicles were also collected and utilized for histopathological analysis. Part of the liver sample was used for determination of antioxidant enzymes such as reduced glutathione (GSH) and catalase (CAT) levels. The rats administered with $AlCl_3$ showed elevated levels of biochemical and hematological parameters and a reduction in levels of sperm count, sperm motility, and oxidative stress parameters, whereas the rats administered with vitamin C/hesperidin (200 and 400 mg/kg) were able to ameliorate $AlCl_3$-induced testicular dysfunction by attenuating $AlCl_3$-induced changes in biochemical and hematological parameters, sperm motility, sperm count, and oxidative stress. Both vitamin C and hesperidin had significant ameliorative effects against $AlCl_3$-induced testicular dysfunction.

Key words: Bioflavonoids, reactive oxygen species, testicular dysfunction, vitamin C

INTRODUCTION

Male infertility factors account for almost half of all occurrences of infertility in 10%–15% of infertile couples. Exposure to heavy metals may have an adverse impact on male fertility, either directly or indirectly. Heavy metals and metal ions can cause oxidative stress and impair antioxidant capability. As a result, testicular tissue is damaged by oxidative stress, which can lead to infertility and poor semen quality.[1] Aluminum (Al) is the most abundant metal in the Earth's crust, and it is commonly used in a wide range of human activities. Al is extensively utilized in several industries, including the packaging of food.[2] Human exposure to Al may be a significant cause of decreasing sperm counts and male fertility.

The provisional tolerable weekly intake of Al for food additives is 2 mg/kg.[2] Al migration occurs when food contacts with Al foil during food packing. As a result, most adults ingest 1–10 mg/day of Al from natural sources.[3] The most

Address for correspondence:
Dr. Subramani Parasuraman,
Unit of Pharmacology, Toxicology and Basic Health Sciences, Faculty of Pharmacy, AIMST University, Bedong, Kedah, Malaysia.
E-mail: parasuraman@aimst.edu.my/ parasuphd@gmail.com

Submitted: 03-Oct-2023 **Revised:** 11-Apr-2024
Accepted: 21-Apr-2024 **Published:** 22-Jul-2024

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/JAPTR
	DOI: 10.4103/JAPTR.JAPTR_458_23

How to cite this article: Parasuraman S, Huey LY, Jee YS. Effect of hesperidin in the prevention of aluminum chloride-induced testicular dysfunction in rats. J Adv Pharm Technol Res 2024;15:194-9.

common way that humans are exposed to Al is through their digestive systems, and the rate of absorption in this area is approximately 0.2%. Through receptor-mediated transferrin endocytosis, Al (or around 0.005% of Al-protein complexes) can reach the nervous system, accumulate in the brain, and cause neurodegeneration.[4] Al also exhibits prooxidant effects and increases the risk of neurodegenerative disorders.[5] Chronic Al exposure reduces the antioxidant enzymes and influences prooxidant systems.[6] The toxicological properties of Al include hepatotoxicity, neurodegenerative disorders, microcytic hypochromic anemia, reproductive toxicity, and genotoxicity. These effects are due to the oxidative degradation of deoxyribonucleic acid and the production of free radicals.[7]

Plants are a major source of medicine for early drug discovery, and they have been used for centuries to treat a wide range of illnesses. Plants synthesize secondary metabolites and these substances play significant roles in the defense, growth, and development of the plant but are not necessary for the essential growth and development of the plant. The biological effects of these secondary metabolites on plants and other living things are diverse. Nearly 50,000 secondary metabolites have been found in plants including hesperidin.[8]

Hesperidin is a bioflavonoid and is abundantly found in citrus fruits (Rutaceae family). Hesperidin is known for its antioxidant activity and exhibits several pharmacological actions against systemic diseases.[9] Hesperidin's effect on testicular dysfunction has not been extensively studied. Hence, the present study is aims to investigate the effect of hesperidin on aluminium chloride ($AlCl_3$)-induced testicular dysfunction in rats.

METHODS

Effect of hesperidin on aluminum chloride-induced testicular dysfunction

Healthy, adult, male Sprague-Dawley (SD) with a weight of 210 ± 15 g were used in the study. The animals were divided into six groups, namely vehicle (normal control), $AlCl_3$, $AlCl_3$ + vitamin C (200 mg/kg), $AlCl_3$ + hesperidin 50 mg/kg, $AlCl_3$ + hesperidin 100 mg/kg, and $AlCl_3$ + hesperidin 200 mg/kg administered groups. The animal doses of hesperidin and vitamin C were based on the scientific literature.[10,11] Testicular dysfunction was induced by administering an intraperitoneal injection of $AlCl_3$ (20 mg/kg) (dissolved in water).[8] Both vitamin C and hesperidin were suspended in carboxymethyl cellulose and administered orally once per day for 21 days. $AlCl_3$ was administered after 1 h of vitamin C/hesperidin administration. Body weight changes were monitored regularly.

At the end of the study, the animals were anesthetized using diethyl ether, and the blood sample was collected through retro-orbital plexus puncture and used for the analysis of biochemical (using a Reflotron Plus biochemical analyzer [Roche Diagnostics, Germany]) and hematological (performed by Gribbles Pathology Lab Sdn. Bhd., Malaysia) parameters. Then, bilateral orchiectomy was carried out to remove both testicles from the animals. Semen was extracted from the right cauda epididymis and used for the determination of sperm count and motility.[12] Finally, part of the liver sample was collected and stored at −20°C until used for determination of antioxidant enzymes such as reduced glutathione (GSH) and catalase (CAT) levels using Ellman's reagent method and hydrogen peroxide method, respectively.[13] Later, organs including the liver, kidney, and testicles were also collected, preserved in 10% neutral formalin, and utilized for histopathological analysis.[14]

Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Analysis was carried out using one-way ANOVA followed by Turkey's multiple comparisons *post-hoc* test. $P \leq 0.05$ was considered statistically significant.

RESULTS

In the present study, reduction in body weight was observed in the $AlCl_3$-administered group, but the results were not significant [Figure 1]. The effect of hesperidin on biochemical and hematological parameters in rats administered with $AlCl_3$ is summarized in Tables 1 and 2. The rats administered with $AlCl_3$ showed a significant increase in the levels of aspartate transaminase, alanine aminotransferase, alkaline phosphatase, total bilirubin, urea, creatinine, neutrophils, and lymphocytes and a significant decrease in the level of albumin and platelets when compared to the control group, whereas both vitamin C and hesperidin 100 and 200 mg/kg prevented the $AlCl_3$-induced changes in the biochemical and hematological parameters.

In sperm analysis, $AlCl_3$-administered animals showed a significant reduction in motility and number of sperm cells

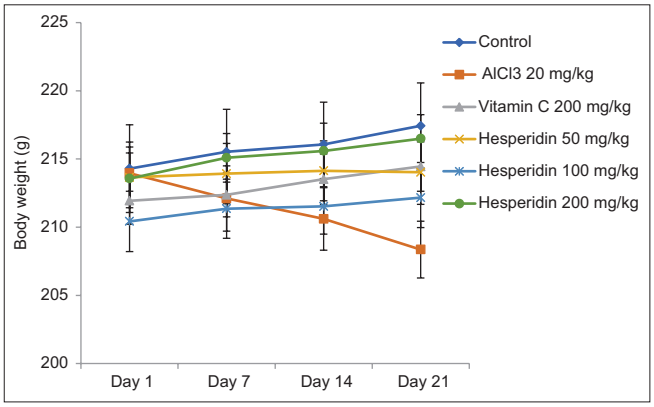

Figure 1: Effect of hesperidin on body weight of experimental rats. All the values are mean ± standard error of the mean ($n = 6$). $AlCl_3$: Aluminum chloride

Table 1: Effect of hesperidin on biochemical parameter

Group	Total protein (g/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	Albumin (g/L)	Globulin (g/L)	Total bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Control	75.50±2.35	88.17±3.52	45.17±2.65	103.83±4.67	44.50±2.45	29.50±2.93	0.93±0.08	22.83±1.40	0.25±0.02
$AlCl_3$ 20 mg/kg	57.00±5.80	121.33±6.13**	82.83±5.28***	136.67±5.53**	30.17±3.03**	25.33±4.30	1.53±0.16***	42.83±4.15***	0.55±0.03***
Vitamin C 200 mg/kg	70.17±3.86	94.50±2.25$^{\#}$	51.83±3.11$^{\#\#\#}$	109.50±9.38$^{\#}$	44.17±2.52$^{\#\#}$	25.33±1.54	0.92±0.06$^{\#\#\#}$	24.50±2.20$^{\#\#\#}$	0.29±0.01$^{\#\#\#}$
Hesperidin 50 mg/kg	62.83±2.34	113.17±8.83*	68.00±7.80*	124.33±4.70	35.67±1.76	24.17±1.45	1.13±0.08$^{\#}$	33.00±3.97	0.35±0.02$^{\#\#\#}$
Hesperidin 100 mg/kg	65.33±5.00	95.50±5.34$^{\#}$	55.50±4.88$^{\#\#}$	111.17±3.15	42.83±3.47$^{\#}$	22.17±2.09	0.97±0.05$^{\#\#}$	27.50±2.26$^{\#\#}$	0.35±0.02$^{\#\#\#}$
Hesperidin 200 mg/kg	69.83±4.96	95.33±3.37$^{\#}$	52.83±2.91$^{\#\#}$	110.00±6.29$^{\#}$	43.33±1.28$^{\#}$	25.50±2.93	0.97±0.08$^{\#\#}$	25.17±1.76$^{\#\#}$	0.31±0.04$^{\#\#\#}$

*$P<0.05$, **$P<0.01$, and ***$P<0.001$ compared with that of the control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ compared with that of $AlCl_3$ 20 mg/kg administered group. All the values are mean±SEM (n=6). SEM: Standard error of the mean, ALT: Alanine aminotransferase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, $AlCl_3$: Aluminum chloride

when compared to control animals, whereas vitamin C/hesperidin restored the motility of sperm cells and sperm count [Figures 2 and 3].

In the antioxidant assay, the animal administered with $AlCl_3$ showed significant decreases in the levels of GSH and CAT when compared to the control group [Figures 4 and 5], whereas vitamin C/hesperidin restored the levels of GSH and CAT [Figures 4 and 5].

In histopathological analysis, the liver of the control group and $AlCl_3$ + Vitamin C/hesperidin 200 mg/kg administered animals showed normal configuration of hepatocytes. The liver of $AlCl_3$-administered animals showed binucleated hepatocytes, mild sinusoidal congestion, and mild centrilobular necrosis [Figure 6]. The liver of animals co-administered with hesperidin 50 or 100 mg/kg administered animals showed binucleated hepatocytes and mild sinusoidal congestion [Figure 6]. $AlCl_3$-administered showed mild vacuolar degeneration of tubules in the kidney [Figure 7]. The kidneys of the control group and $AlCl_3$ + vitamin C or hesperidin 50/100/200 mg/kg showed normal renal cells [Figure 7]. These findings indicated hesperidin possesses a nephroprotective effect at concentrations of 50 mg/kg and above. The testis of the control group and $AlCl_3$ + Vitamin C/hesperidin 200 mg/kg administered animals showed normal seminiferous tubule morphology (the testis is surrounded by a thick tunic of connective tissue) [Figure 8]. The testis of the $AlCl_3$-administered animals shows oligospermia and increased spaces between tubules. The testis of the $AlCl_3$ + hesperidin 50/100 mg/kg administered animals show increased spaces between tubules [Figure 8].

DISCUSSION

In the present study, hesperidin prevented the $AlCl_3$-induced testicular dysfunction by attenuating $AlCl_3$-induced changes in biochemical and hematological parameters, sperm

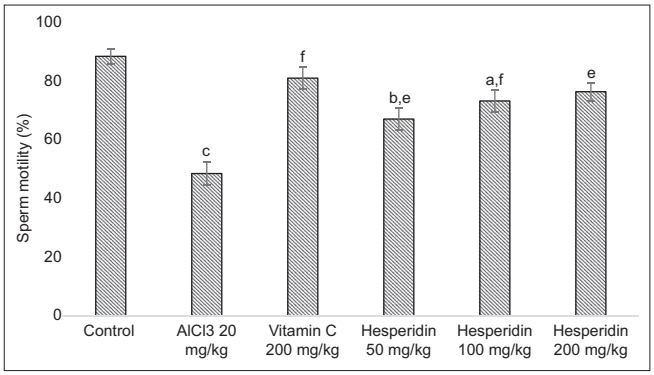

Figure 2: Effect on sperm motility. All the values are mean ± standard error of the mean (n = 6). $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$ compared with that of the control group; $^{e}P < 0.01$ and $^{f}P < 0.001$ compared with that of $AlCl_3$ 20 mg/kg administered group. $AlCl_3$: Aluminum chloride

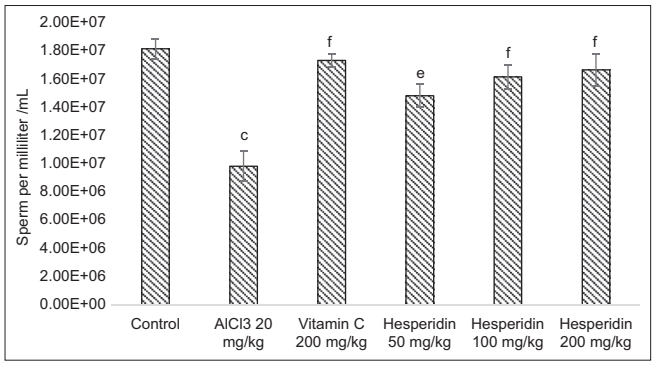

Figure 3: Effect of hesperidin on sperm count. All the values are mean ± standard error of the mean ($n = 6$). $^{c}P < 0.001$ compared with that of the control group; $^{c}P < 0.01$ and $^{f}P < 0.001$ compared with that of $AlCl_3$ 20 mg/kg administered group. $AlCl_3$: Aluminum chloride

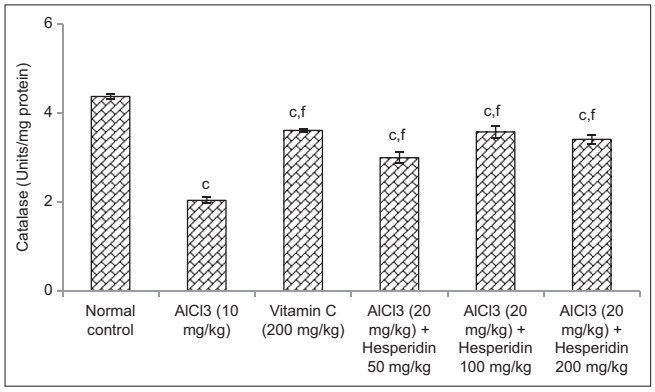

Figure 5: Effect of hesperidin on catalase enzyme. All the values are mean ± standard error of the mean ($n = 6$). $^{c}P < 0.001$ compared with that of the control group; $^{f}P < 0.001$ compared with that of $AlCl_3$ 20 mg/kg administered group. $AlCl_3$: Aluminum chloride

motility, sperm count, and oxidative stress. Al has been indicated to induce male reproductive toxicity by various mechanisms including an increase in the oxidative stress enzyme levels, affecting the endocrine system, impairment of the blood–testis barrier, alteration in membrane function, changes to spermatogenesis, and changes in steroidogenesis.[15] Al also causes a dose-dependent reduction in sperm viability, motility, and increased malondialdehyde (MDA) level.[16]

In the present study, a decrease in body weight (results were not significant) was observed in the $AlCl_3$-administered group, and this may be due to the anorectic effect of Al.[17] In the biochemical analysis, $AlCl_3$-administered rats showed elevated levels of liver and renal markers which indicate that animals are at risk of liver and kidney damage. In experimental animals, $AlCl_3$ disturbs the mitochondrial Deoxyribonucleic acid (DNA) transcript and mitochondrial energy metabolism and causes an increase in liver enzymes.[18] $AlCl_3$-induced nephrotoxicity is due to the accumulation of Al in the renal system.[19] Chronic Al exposure causes oxidative stress and suppresses liver and kidney functions.

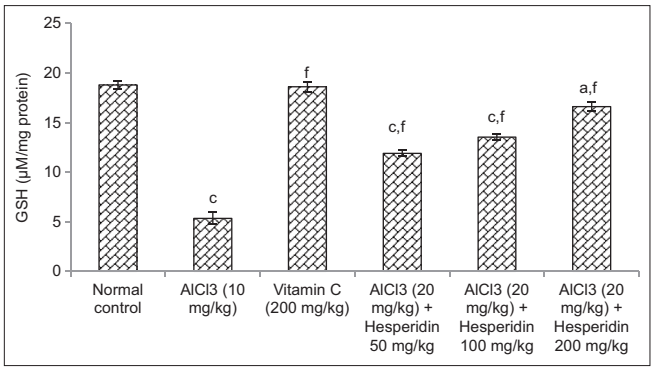

Figure 4: Effect of hesperidin on reduced glutathione enzyme. All the values are mean ± standard error of the mean ($n = 6$). $^{c}P < 0.001$ and $^{a}P < 0.05$ compared with that of the control group; $^{f}P < 0.001$ compared with that of $AlCl_3$ 20 mg/kg administered group. $AlCl_3$: Aluminum chloride

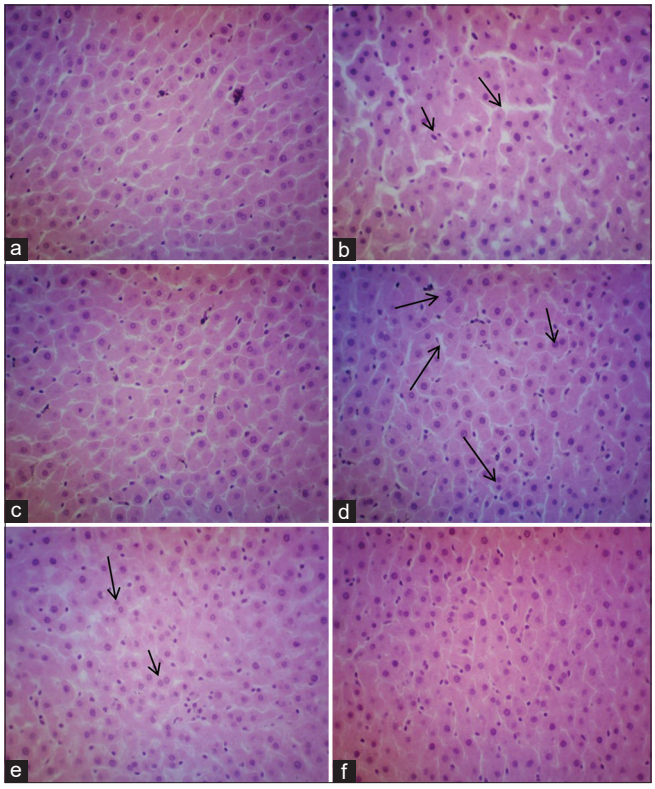

Figure 6: Histopathology of liver in rats (photomicrograph of hematoxylin and eosin; x400) (a) Control; (b) $AlCl_3$ 20 mg/kg; (c) Vitamin C 200 mg/kg; (d) Hesperidin 50 mg/kg; (e) Hesperidin 100 mg/kg; (f) Hesperidin 200 mg/kg

In the present study, vitamin C and hesperidin prevented $AlCl_3$-induced changes in sperm count and motility. The ameliorative of hesperidin is based on its ability to scavenge free radicals. The excessive reactive oxygen species may disrupt mitochondrial energy production which leads to a significant increase in total abnormal sperm rate and decreases sperm motility.[20] Al can also cross the blood–testis barrier and cause oxidative stress, which affects the testes' cellular membranes and interferes with spermatogenesis.[21]

Table 2: Effect of hesperidin on hematological parameter

Parameter	Control	$AlCl_3$ 20 mg/kg	Vitamin C 200 mg/kg	Hesperidin 50 mg/kg	Hesperidin 100 mg/kg	Hesperidin 200 mg/kg
Hemoglobin (g/L)	131.83±7.47	99.50±6.79	126.50±10.48	112.17±12.63	119.83±4.09	126.17±6.83
Red blood cell count ($\times10^{12}$/L)	4.85±0.32	4.17±0.22	4.75±0.17	4.37±0.23	4.73±0.17	4.80±0.15
Platelet count ($\times10^9$/L)	985.83±45.30	818.17±48.86*	985.33±24.75$^{\#}$	899.00±40.37	962.00±35.69	982.00±28.29
Total white blood cell ($\times10^9$/L)	9.35±0.40	10.68±0.52	9.13±0.41$^{\#}$	9.32±0.27	9.13±0.18$^{\#}$	9.28±0.26
Neutrophils ($\times10^9$/L)	0.85±0.03	1.17±0.09***	0.89±0.04$^{\#\#}$	0.98±0.03	0.91±0.03$^{\#\#}$	0.86±0.02$^{\#\#\#}$
Lymphocytes ($\times10^9$/L)	10.57±0.22	11.70±0.25*	10.38±0.27$^{\#\#}$	10.38±0.24$^{\#\#}$	10.32±0.21$^{\#\#}$	10.83±0.11
Monocytes ($\times10^9$/L)	1.31±0.06	1.44±0.06	1.27±0.06	1.28±0.07	1.34±0.03	1.29±0.02
Eosinophils ($\times10^9$/L)	0.11±0.01	0.12±0.01	0.11±0.01	0.12±0.01	0.11±0.01	0.11±0.01

*$P<0.05$ and ***$P<0.001$ compared with that of the control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$ compared with that of $AlCl_3$ 20 mg/kg administered group. All the values are mean±SEM ($n=6$). $AlCl_3$: Aluminum chloride, SEM: Standard error of the mean

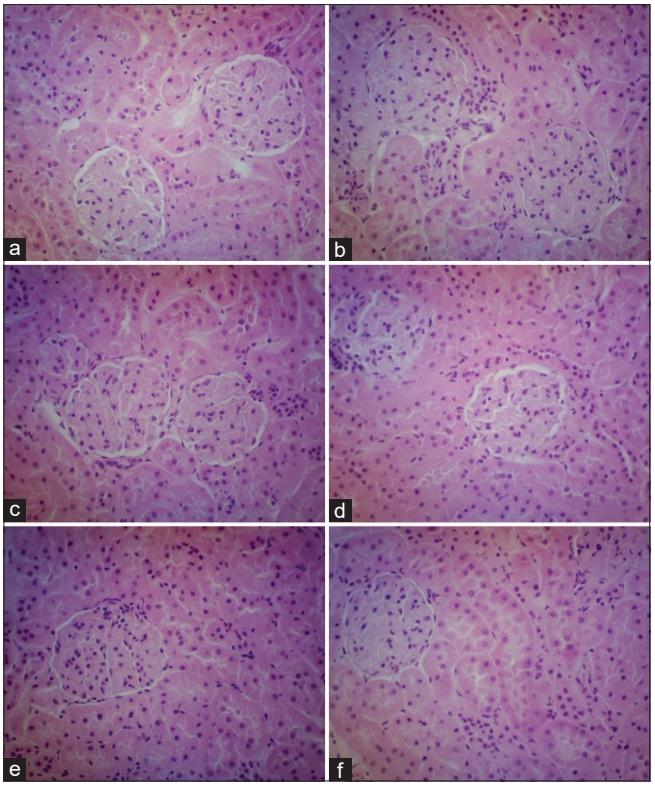

Figure 7: Histopathology of kidney in rats (photomicrograph of haematoxylin and eosin; x400) (a) Control; (b) $AlCl_3$ 20 mg/kg; (c) Vitamin C 200 mg/kg; (d) Hesperidin 50 mg/kg; (e) Hesperidin 100 mg/kg; (f) Hesperidin 200 mg/kg

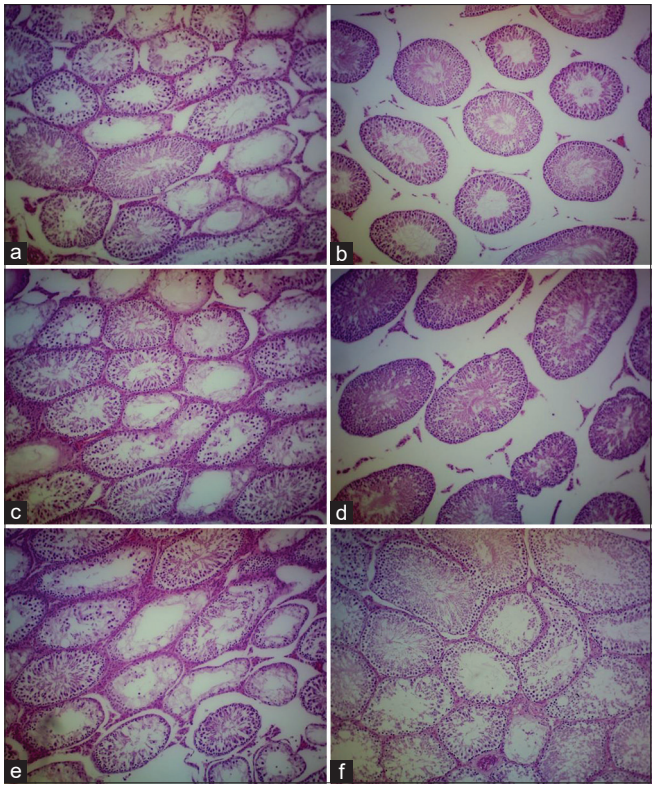

Figure 8: Histopathology of testis in rats (photomicrograph of hematoxylin and eosin; x100) (a) Control; (b) $AlCl_3$ 20 mg/kg; (c) Vitamin C 200 mg/kg; (d) Hesperidin 50 mg/kg; (e) Hesperidin 100 mg/kg; (f) Hesperidin 200 mg/kg

$AlCl_3$-administered rats also showed decreases in the levels of GSH and CAT. Ogunlade *et al*. also found decreased antioxidant enzymes and increased MDA levels in $AlCl_3$-administered animals.[22] The reduction in the antioxidant enzyme activities demonstrated an impaired antioxidant system, leading to oxidative stress-induced testicular dysfunction/toxicity. However, vitamin C/hesperidin ameliorates $AlCl_3$-induced oxidative stress by increasing antioxidant enzyme levels. It has been inferred that vitamin C/hesperidin prevents testicular dysfunction by scavenging free radicals and lowering their levels. In a clinical study, hesperidin is able to reduce the level of oxidative stress markers by increasing antioxidant enzyme levels.[23] During the cryopreservation-thawing process, hesperetin (a metabolite of hesperidin) improves the quality of human sperm (viability and motility) and also protects sperm from reactive oxygen species, lipid peroxidation, and apoptosis.[24]

The findings of histopathological analysis indicate hesperidin possesses hepatoprotective, nephroprotective, and testicular protective effects against $AlCl_3$-induced tissue damage. In histopathological analysis, $AlCl_3$-administered animals showed mild-to-moderate tissue damage. The

histopathology of the liver, kidney, and testis of animals in the control group, $AlCl_3$ + vitamin C, and $AlCl_3$ + hesperidin 100/200 mg/kg showed minimal histological changes or normal cellular arrangement. Al is known to exhibit hepatotoxicity, nephrotoxicity, and testicular damage.[18,25,26]

The present study data indicated that $AlCl_3$-inducing testicular dysfunction by increasing oxidative stress enzymes and co-administration of hesperidin prevents the $AlCl_3$-induced testicular dysfunction.

CONCLUSION

$AlCl_3$ caused testicular dysfunctions in experimental rats. The rats co-administered with vitamin C/hesperidin (200 and 400 mg/kg) were able to ameliorate $AlCl_3$-induced changes in biochemical and hematological parameters and prevented $AlCl_3$-induced testicular dysfunction in experimental animals. Both vitamin C and hesperidin exhibited antioxidant activity, and inhibition of $AlCl_3$-induced changes may be mediated through their antioxidant effect.

Ethics approval

Prior to the study, permission was obtained from the AIMST University Human and Animal Ethics Committee to carry out animal experiments (AUAEC/FOP/2021/05).

Financial support and sponsorship

The authors received financial support for publication of this article from AIMST University.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Abu Khudir R, Almutairi HH, Abd El Rahman SS, El Said KS. The palliative and antioxidant effects of hesperidin against lead-acetate-induced testicular injury in male wistar rats. Biomedicines 2023;11:2390.
2. El Daouk S, Pineau A, Taha M, Ezzeddine R, Hijazi A, Al Iskandarani M. Aluminum exposure from food in the population of Lebanon. Toxicol Rep 2020;7:1025-31.
3. Alasfar RH, Isaifan RJ. Aluminum environmental pollution: The silent killer. Environ Sci Pollut Res Int 2021;28:44587-97.
4. Bondy SC. Low levels of aluminum can lead to behavioral and morphological changes associated with Alzheimer's disease and age-related neurodegeneration. Neurotoxicology 2016;52:222-9.
5. Esparza JL, Gómez M, Romeu M, Mulero M, Sánchez DJ, Mallol J, *et al.* Aluminum-induced pro-oxidant effects in rats: Protective role of exogenous melatonin. J Pineal Res 2003;35:32-9.
6. Skalny AV, Aschner M, Jiang Y, Gluhcheva YG, Tizabi Y, Lobinski R, *et al.* Molecular mechanisms of aluminum neurotoxicity: Update on adverse effects and therapeutic strategies. Adv Neurotoxicol 2021;5:1-34.
7. Al Rekabi BK. The protective role of taurine and *Zingiber officinale* on aluminum chloride induced testicular injury and impaired spermatogenesis in adults male rabbits: Biochemical, hormonal and histopathological study. Plant Arch 2019;19:755-67.
8. Teoh ES. Secondary metabolites of plants. In: Medicinal Orchids of Asia. Cham: Springer International Publishing; 2016. p. 59-73. Available from: https://www.link.springer.com/10.1007/978-3-319-24274-3_5. [Last accessed on 2023 Aug 25].
9. Pyrzynska K. Hesperidin: A review on extraction methods, stability and biological activities. Nutrients 2022;14:2387.
10. Parasuraman S, Thinagaran JN. Evaluation of the antidiabetic activity of hesperidin on streptozotocin-induced diabetes mellitus in Swiss albino mice. Free Radic Antioxid 2023;13:46-9.
11. Giordano V, Albuquerque RP, do Amaral NP, Chame CC, de Souza F, Apfel MÍ. Supplementary Vitamin C does not accelerate bone healing in a rat tibia fracture model. Acta Ortop Bras 2012;20:10-2.
12. Cheraghi E, Golkar A, Roshanaei K, Alani B. Aluminium-induced oxidative stress, apoptosis and alterations in testicular tissue and sperm quality in Wistar rats: Ameliorative effects of curcumin. Int J Fertil Steril 2017;11:166-75.
13. Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J 2013;21:143-52.
14. Parasuraman S, Ching TH, Leong CH, Banik U. Antidiabetic and antihyperlipidemic effects of a methanolic extract of *Mimosa pudica* (fabaceae) in diabetic rats. Egypt J Basic Appl Sci 2019;6:137-48.
15. Pandey G, Jain GC. A review on toxic effects of aluminium exposure on male reproductive system and probable mechanisms of toxicity. Int J Toxicol Appl Pharmacol 2013;3:48-57.
16. Yokel RA. Aluminum reproductive toxicity: A summary and interpretation of scientific reports. Crit Rev Toxicol 2020;50:551-93.
17. Bekhedda H, Menadi N, Demmouche A, Ghani A, Mai H. Histological study of the effects of aluminum chloride exposure on the brain of Wister rats female. J Drug Deliv Ther 2020;10:37-42.
18. Xu F, Liu Y, Zhao H, Yu K, Song M, Zhu Y, *et al.* Aluminum chloride caused liver dysfunction and mitochondrial energy metabolism disorder in rat. J Inorg Biochem 2017;174:55-62.
19. Obafemi TO, Anyalechi DI, Afolabi BA, Ekundayo BE, Adewale OB, Afolabi OB, *et al.* Nephroprotective effects of gallic acid and hesperidin in aluminum chloride-induced toxicity in rats. Phytomed Plus 2022;2:100378.
20. Afolabi OK, Wusu AD, Ugbaja R, Fatoki JO. Aluminium phosphide-induced testicular toxicity through oxidative stress in Wistar rats: Ameliorative role of hesperidin. Toxicol Res Appl 2018;2:1-11.
21. Hala AH, Khattab ZA, Abdallah G, Kamel M. Grape seed extract alleviate reproductive toxicity caused by aluminium chloride in male rats. J Am Sci 2010;6:352-61.
22. Ogunlade B, Adelakun S, Iteire K. Sulforaphane response on aluminum-induced oxidative stress, alterations in sperm characterization and testicular histomorphometry in Wistar rats. Int J Reprod Biomed 2020;18:611-24.
23. Celik E, Oguzturk H, Sahin N, Turtay MG, Oguz F, Ciftci O. Protective effects of hesperidin in experimental testicular ischemia/reperfusion injury in rats. Arch Med Sci 2016;12:928-34.
24. Valipour J, Mojaverrostami S, Abouhamzeh B, Abdollahi M. Protective effects of hesperetin on the quality of sperm, apoptosis, lipid peroxidation, and oxidative stress during the process of cryopreservation: An experimental study. Int J Reprod Biomed 2021;19:35-46.
25. Mohammed HH, Abd Ali IK, Reshag AF. Histological changes in the liver, kidney and spleen of white albino rat after aluminum chloride administration. Iraqi J Vet Med 2017;41:1-6.
26. Lokman M, Ashraf E, Kassab RB, Abdel Moneim AE, El Yamany NA. Aluminum chloride-induced reproductive toxicity in rats: The protective role of zinc oxide nanoparticles. Biol Trace Elem Res 2022;200:4035-44.