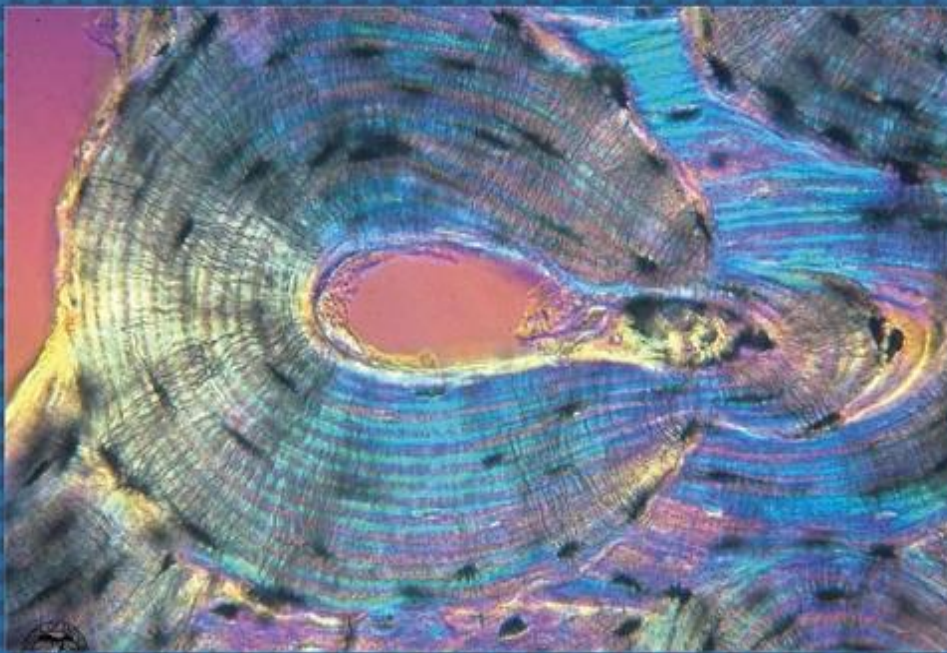




EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
HISTOLOGY & HISTOCHEMISTRY

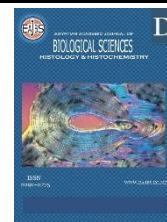
D



ISSN
2090-0775

WWW.EAJBS.EG.NET

Vol. 15 No. 2 (2023)



Histological and Ultrastructural Studies on the Protective Effects of *Chlorella vulgaris* on Healthy Testis against Toxicity of The Therapeutic Regimen of Cisplatin

Ebtehal G. Abdelghaffar*; Hany A. Hafney; Hala M. Ebaid and
Heba N. Gad EL-Hak

Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

E.Mail* : ebtehal.gameel@gmail.com

ARTICLE INFO

Article History

Received:19/8/2023

Accepted:23/ 9/2023

Available:26/9/2023

Keywords:

Cisplatin; Chlorella vulgaris; Testis; spermatogenesis; ultrastructure

ABSTRACT

A typical chemotherapy agent used to treat a number of tumors is called cisplatin. Male infertility is one of the side effects of cisplatin therapy, which is one of the drugs' side effects. Male mammalian germ cells have been shown to be protected by *Chlorella vulgaris* supplementation. The goal of the current study was to examine, using light and electron microscopy, the protective effects of *Chlorella vulgaris* supplementation against damage to healthy Testis tissue caused by the cisplatin-based treatment regimen. Each of the four groups, which each had five rats, contained the animals. Saline injections intraperitoneally were given to the animals in the control group. Cisplatin family: The same dose of cisplatin utilized in the human treatment procedure, 134 mg/kg intraperitoneally, was administered to the animals for three months. For three months, animals in the *Chlorella* group consumed 150 mg/kg of *Chlorella* daily. *Chlorella/Cisplatin* group: The rats got 150 mg/kg of *Chlorella* orally daily for three months and 134 mg/kg of cisplatin intraperitoneally once each week. Following cisplatin treatment, abnormalities in the testis were averted by *Chlorella* supplementation, according to the results of light and transmission ultrastructure microscopy research.

INTRODUCTION

One cancer treatment approach that can be used alone or in combination with other methods, such as surgery and radiotherapy, is chemotherapy (Rampling *et al.*, 2004).

One of the most widely used chemotherapeutic medications, cisplatin, has persisted (A stolfi *et al.*, 2013).

Among the tumor types that it is successful against include solid tumors, hematological malignancies, bladder, head and neck, esophageal, gastric, pulmonary, testicular, and ovarian cancers, lymphoma, and

Osteosarcoma (Boulikas and Vougiouka, 2004). Despite having effective anticancer properties, the drug cisplatin also affects the testes in a number of harmful and toxic ways (Chabner *et al.*, 2006). As a result, there is great interest in combining anticancer drugs with herbal treatments to boost their potency while lowering systemic toxicity by using lower drug doses (Abdallah *et al.*, 2019).

Chlorella vulgaris a very nutritious unicellular freshwater microalga and is full of nutrients like proteins, carbs, vitamins, antioxidants, fatty acids, and trace minerals (Priyadarshani and Rath, 2012).

Chlorella vulgaris is commonly utilized as a functional food or a magical supplement for people because of its abundant component. Its advantageous impacts on human health have been proven (Vartak *et al.*, 2022).

We thus focused on investigating the induced testis injury by cisplatin on healthy tissue along with the potential use of *Chlorella vulgaris* as an ameliorative agent to overcome these injuries. To achieve such an objective, the male sex hormone and testicular tissue of rats assessed by performing histopathological and ultrastructure examinations.

MATERIALS AND METHODS

Experimental Groups and Design of Work:

The animals were placed into four groups, each with five rats, after a one-week acclimation period. Saline (NaCl) was administered intraperitoneally to the rats in the control group. Cisplatin group: For three months, the animals received 134 mg/kg of cisplatin intraperitoneally, which is the same amount as was utilized in the human treatment procedure. *Chlorella* group: According to Zainul Azlan *et al.* (2020), the animals got 150 mg/kg of *chlorella* orally every day for three months. Cisplatin/*Chlorella* group: Over the course of three months, the rats received 134 mg/kg of cisplatin intraperitoneally once per week and 150 mg/kg of *chlorella* orally daily.

Blood and Testes Sampling:

Under anesthesia with (100 mg/Bwt) thiopental sodium, blood samples were taken from the medial retro-orbital venous plexus of rats using capillary tubes (Micro Hematocrit Capillaries) (Abdi-Azar and Maleki, 2014). Following the blood sample, the animals were dislocated to cause death, and the testis was removed for histology and ultrastructure analysis.

Male Sex Hormone:

According to Dada and Nduka (1980), testosterone was found in the

blood serum using Elisa kits (MyBioSource company) (catalog no. MBS580035).

Histological Preparation:

The testis of each animal was rapidly removed when they were slaughtered, rinsed with normal saline, and preserved in 10% neutral formaldehyde. The samples were dehydrated, cleaned in terpineol for two days, rinsed in benzene for ten minutes, and then embedded in three different layers of pure paraffin wax. A microtome was used to cut sections of around 5 microns thickness, which were then placed on spotless glass slides for histological preparation. Hematoxylin and Eosin (H&E) were used to stain a piece of the tissue. The slides were then mounted with DPX and cleaned with xylene (Fischer *et al.*, 2008).

Electron Microscopy Analysis:

Under a dissecting microscope, little testicular fragments were removed and sliced in the presence of 2% glutaraldehyde. Specimens were placed in a solution containing 2% glutaraldehyde fixative and 0.1 M sodium cacodylate buffer for 24 hours. They were then rinsed in 0.1 M phosphate buffer at 4 °C and post-fixed with 1% osmium tetroxide. The tissues were then injected with resin following dehydration in a progressive series of ethyl alcohol. On the ultramicrotome (Leica Ultracut UCT, Heidelberg, Germany), portions of tissue-containing blocks were cut into semi-thin layers. The transmission electron microscope was used to analyze ultrathin slices that had been stained with lead citrate and uranyl acetate (Sato *et al.*, 2008).

Statistical Analysis of Data:

The testosterone data was expressed in mean \pm standard error (SE) and analyzed using SPSS 11.0 for Windows. The statistical significance of differences when *p*-value less than 0.05 was evaluated by using a one-way analysis of variance (ANOVA) followed

by post-hoc Duncan to make the comparison between means.

RESULTS

Effect of Cisplatin and Chlorella Supplement on The Testosterone of Rats:

For the control and various treated groups, serum testosterone values are estimated. In terms of data compared to the control group, the chlorella and chlorella/cisplatin groups both significantly outperformed the cisplatin group. Figure (1).

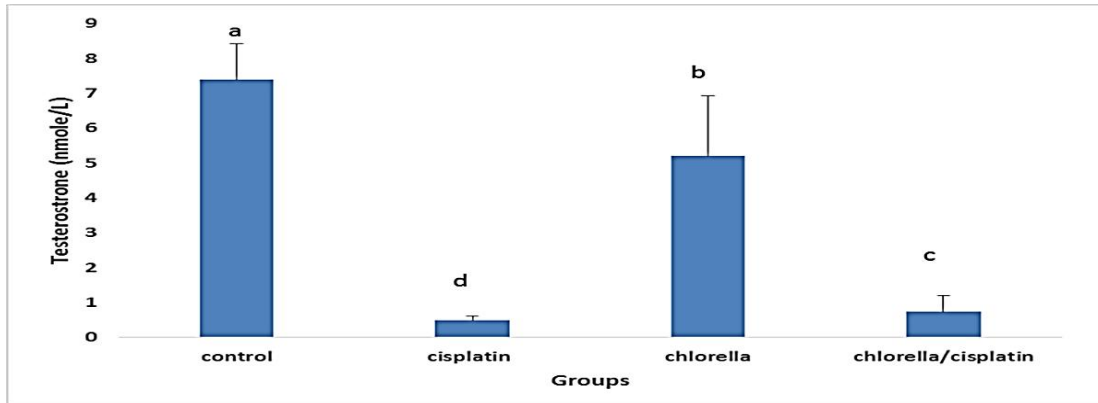


Fig. 1: Effect of Cisplatin and Chlorella supplement treatment on testosterone. Data were expressed as means \pm SEM, $n=5$. Data were statically analyzed using One-way ANOVA followed by Duncan multiple comparisons test. Different letters showed data from the different rows, which is statistically significant, $P \leq 0.05$.

Histological Examination:

Rats treated with chlorella and control rats with examined testicles both displayed normal histological structures Figure 2(a&b). The tunica albuginea, a capsule of connective tissue, appeared to surround the testis. Seminiferous tubules with uniform contours that resembled spherical or oval shapes made up the testicular parenchyma. There is fragile loose connective tissue and Leydig cells in the interstitial spaces between the tubules. A basement membrane surrounding myoid cells and lined with stratified spermatogenic cells and supporting Sertoli cells encloses the seminiferous tubules. The seminiferous tubules are typically organized in several series of spermatogenic layers and

spermatozoa in control and chlorella-treated rats. In the lumen of every seminiferous tubule, spermatogonia continued to develop into adult spermatozoa. Rat testes that had received cisplatin treatment Seminiferous tubules in Figure 2 (c) were severely degenerating and atrophic, and the lumen of the tubules included spermatozoa debris and hypocellular spermatogenic cells. Moreover, the lumen of the majority of tubules lacked free mature spermatozoa. Figure 2(d) illustrates the typical appearance of the germinal epithelium and the spermatogenic cells with the regular arrangement of the seminiferous tubules in rats that had been given cisplatin and chlorella treatment.

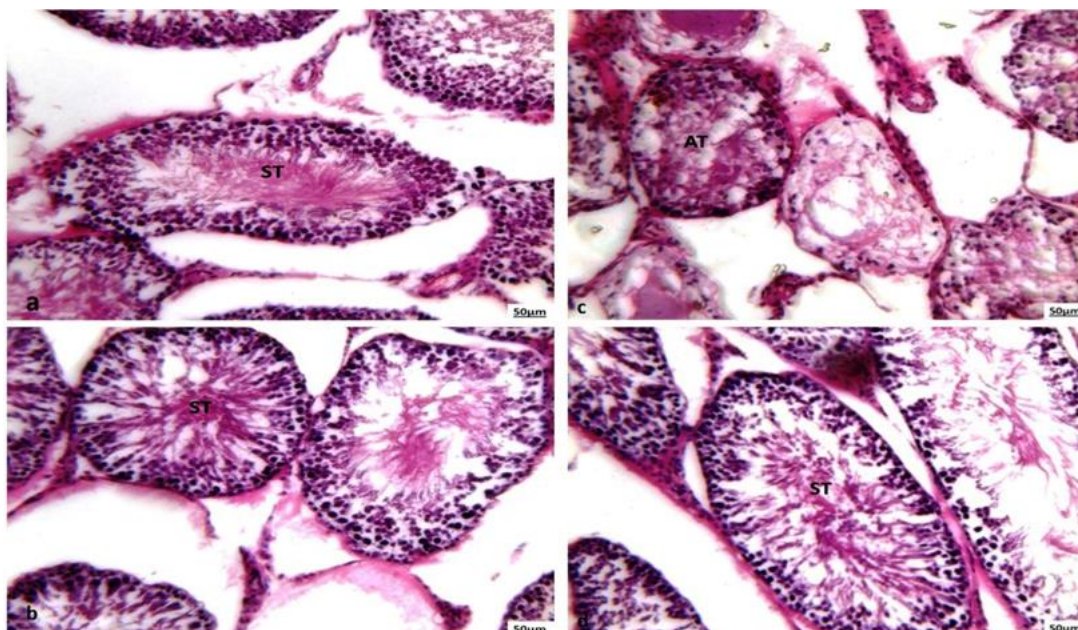


Fig.2: Photomicrographs of the testis of (a) control rats and (b) treated rats with chlorella showed normal seminiferous tubules (ST) with different spermatogenic stages. (c) treated rats with cisplatin showed atrophic seminiferous tubules (AT) devoid of the spermatogenic stage, and free mature spermatozoa were absent in the lumen of most tubules. (d) treated rats with chlorella and cisplatin showed normal seminiferous tubules (ST) with different spermatogenic stages. (H & E×200).

Ultrastructure Examination:

Ultrastructure examination of the testis sections of the control and chlorella groups Figure 3 (a&b) showed that the seminiferous tubule appeared to be surrounded by a basement membrane and myoid cell. The basement membrane was regular and of uniform pattern; the myoid cell was slender, smooth muscle-like, and was present outside the basement membrane within the basal lamina. The spermatogonia were rounded and rested on the basement membrane containing rounded euchromatic nuclei and prominent nucleoli, and many mitochondria were observed scattered within the cytoplasm. The 1ry spermatocytes appeared to be large cells with large euchromatic nuclei. Spermatids appeared with large euchromatic nuclei and its characteristic acrosomal vesicle; many sperms or fully formed spermatozoa were also detected embedded within the Sertoli cell, which appeared as a long dark cell with an irregular outline. Sections of mature sperm revealed the normal structure of middle pieces of sperms, which were formed of a central axoneme surrounded

by a fibrous sheath, then a mitochondrial sheath, and covered by a plasma membrane. The end piece of the tail was formed of axoneme only. Sertoli cells, with their cytoplasm, extend from the basal lamina to the lumen of the seminiferous tubule and envelop the adjacent germinal elements. The nucleus is infolded and sometimes has a very irregular shape; the nucleoplasm is homogenous, and the cytoplasm contains abundant endoplasmic reticulum, ovoid Golgi apparatus, and spherical or cylinder-shaped mitochondria.

Ultrastructure examination of the testis sections of the cisplatin group Figure 3 (c) showed that the seminiferous tubule appeared to be surrounded by a thin basement membrane and degenerated myoid cell. The cytoplasm is vacuolated and disrupted, and some of their cells appear ruptured; despite that, the spermatids were recognized by the characteristic acrosomal vesicle surrounded by a widened intracellular bridge. Sertoli cells, with their cytoplasm, the chromatin clumped in their margin, and some of their membranes are ruptured. Ultrastructure

examination of the testis sections of the chlorella/cisplatin group Figure 3 (d)

showed a normal structure like the control group.

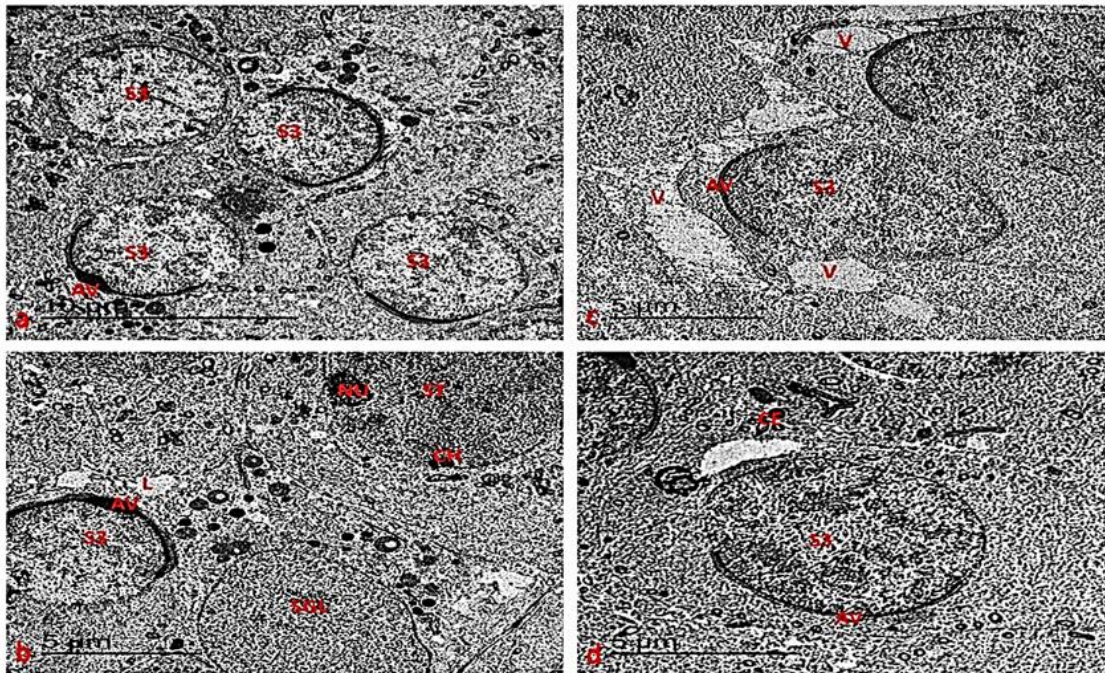


Fig. 3: An electron micrograph of the testis from (a) the control group showed normal ultrastructure of Spermatids (S3) with its large euchromatic nuclei and its characteristic acrosomal vesicle (AV). (b) treated group with chlorella showed normal ultrastructure of Spermatids (S3) with its large euchromatic nuclei and its characteristic acrosomal vesicle (AV). The spermatogonia (SGL) were rounded and rested on the basement membrane, containing rounded euchromatic nuclei within the cytoplasm. Sertoli cells (ST), with their cytoplasm, extend from the basal lamina to the lumen of the seminiferous tubule and envelop the adjacent germinal elements. The nucleus is enfolded and sometimes has a very irregular shape; the nucleoplasm is homogenous, and the cytoplasm contains abundant endoplasmic reticulum ovoid Golgi apparatus, dense chromatin bodies (CH), lipid droplets (L) and spherical or cylinder-shaped mitochondria. (c) treated group with cisplatin showed cytoplasm is vacuolated (V) and disrupted, and some of the cells appeared ruptured; despite that, the spermatids (S3) were recognized by the characteristic acrosomal vesicle (AV) surrounded with a widened intracellular bridge. (d) treated group with chlorella in combination with cisplatin showed normal spermatids (S3) were recognized by the characteristic acrosomal vesicle (AV). The cytoplasm contains lipofusion granules (CE).

DISCUSSION

This study used histopathological analysis and cellular examination of testes tissues to examine the potential ameliorative effects of *Chlorella vulgaris* supplement against the therapeutic regime of cisplatin drug-induced toxicity on the testis of healthy rats. Both healthy spermatogenesis and the preservation of seminiferous tubules' proper structure depend on testosterone (Sharpe *et al.*, 1992). Chlorella treatment effectively reversed the drop in testosterone levels caused by cisplatin, which followed the preceding investigations of Silici *et al.* (2009) and

Osama *et al.* (2019). As one of the potential causes, cisplatin-induced significant damage to Leydig and Sertoli cells due to the increased formation of free radicals could account for this substantial decrease in the hormone level (Ilbey *et al.*, 2009). Additionally, numerous studies suggested that cisplatin may affect testosterone levels by impairing the expression of the luteinizing hormone (LH) receptor and cholesterol mobilization to mitochondrial cytochrome P450, thereby obstructing the initial stages of testosterone production (Essawy *et al.*, 2017). Chlorella's anti-oxidative

function, which guards against OS-induced Leydig cell damage, may account for its favorable impact on testosterone levels (Abdel-khalek *et al.*, 2023). Rats exposed to pollutants or toxins in the laboratory have been evaluated for health using histopathological and ultrastructural alterations as indicators (Jiang *et al.*, 2021). Cisplatin has a toxic reproductive effect (Abdel-Wahab *et al.*, 2020). The present study's testicular injury in cisplatin-treated rats served as a warning sign of the drug's negative effects. In the testis of rats given cisplatin treatment, there were fewer Leydig cells, fewer spermatogenic cells lining seminiferous tubules, and fewer spermatogenic cells themselves. In the lumen of the seminiferous tubules, spermatozoa debris was seen. These changes in the histology of the testis, highly dividing cells, may be due to the fact that cisplatin has been thought to be a powerful inhibitor that prevents cell division (Bunch and Eastman, 1996) or commonly linked to testicular OS induced by cisplatin (Moradi *et al.*, 2021). These findings are in agreement with Bushra and Bastwrous (2022), that observed that during cisplatin treatment, it was seen that the number of spermatogenic cells decreased and the epithelial height of the seminiferous tubules decreased. As opposed to the cisplatin group, rats treated with chlorella plus cisplatin shield the seminiferous tubules from deterioration. In the ultrastructural examination, the testis of treated rats with cisplatin showed evidence of increased intercellular spaces between the various components of the seminiferous tubules, which is indicative of tubular edema (Krishna *et al.*, 2018). In this present study, one of the early and most common morphological features of damage to the testis is vacuolations. These might have formed due to the autophagosomes formed for the phagocytosis of necrotic germ cells by Sertoli cells (Wang and Han, 2019). Another reason for the vacuolization may be due to the swelling

and coalescence of intracellular membrane-bound organelles like the ER (Krishna *et al.*, 2018). Spermatogenic cells and Sertoli cells degenerated in the current investigation. This could be a result of ROS, which was detected in the measured parameter, directly harming either these spermatogenic cells or Sertoli cells. Sertoli cells are crucial to the process of spermatogenesis (Alves *et al.*, 2013). Sertoli cells produce lactate, which is important for the survival of germ cells (Boussouar and Benahmed, 2004). Any injury to Sertoli cells, as found in the present study, would affect the nutrition and sustenance of spermatogenic cells (Li *et al.*, 2009). Additionally, as seen in this work, the separation of Sertoli cells from spermatogenic cells as a result of intercellular edema would hinder the passage of nutrients from Sertoli cells to the spermatogenic cells (El Shafai *et al.*, 2011).

Conclusions

The current study showed that the equivalent therapeutic regime and dose of cisplatin caused adverse changes to the healthy testes of rats. Cisplatin-induced histological and ultrastructural changes of testes. Moreover, Chlorella protects the testis from the damage induced by cisplatin.

REFERENCES

- Abdallah, H.M., Abdel-Rahman, R.F., El Awdan, S.A., Allam, R.M., El-Mosallamy, A.E., Selim, M.S., Mohamed, S.S., Arbid, M.S., and Farrag, A.R.H. (2019). Protective effect of some natural products against chemotherapy-induced toxicity in rats. *Heliyon*, 5, e01590.
- Abdel-khalek, A.-K.E.-S., El-Maghraby, M.M., Elbially, Z.I., Almadaly, E., Shukry, M., El-Badawy, A., Zaghoul, H.K., and Assar, D.A. (2023). Mitigation of endogenous oxidative stress and improving growth, haemato-biochemical parameters, and reproductive performance of Zaraibi goat bucks by dietary

- supplementation with *Chlorella vulgaris* or/and vitamin C. *Tropical Animal Health and Production* (2023) 55:267
- Abdel-Wahab, B.A., Alkahtani, S.A., and Elagab, E.A. (2020). Tadalafil alleviates cisplatin-induced reproductive toxicity through the activation of the Nrf2/HO-1 pathway and the inhibition of oxidative stress and apoptosis in male rats. *Reproductive Toxicology*, 96, 165-174.
- Abdi-Azar, H., and Maleki, S. (2014). Comparison of the anesthesia with thiopental sodium alone and their combination with *Citrus aurantium* L.(Rutaseae) essential oil in male rat. *Bulletin of Environment, Pharmacology and Life Sciences*, 3, 37-44.
- Alves, M.G., Rato, L., Carvalho, R.A., Moreira, P.I., Socorro, S., and Oliveira, P.F. (2013). Hormonal control of Sertoli cell metabolism regulates spermatogenesis. *Cellular and molecular life sciences*, 70, 777-793.
- Astolfi, L., Ghiselli, S., Guaran, V., Chicca, M., Simoni, E., Olivetto, E., Lelli, G., and Martini, A. (2013). Correlation of adverse effects of cisplatin administration in patients affected by solid tumours: A retrospective evaluation. *Oncology reports*, 29, 1285-1292.
- Boulikas, T., and Vougiouka, M. (2004). Recent clinical trials using cisplatin, carboplatin and their combination chemotherapy drugs. *Oncology reports*, 11, 559-595.
- Boussouar, F., and Benahmed, M. (2004). Lactate and energy metabolism in male germ cells. *Trends in Endocrinology & Metabolism*, 15, 345-350.
- Bunch, R.T., and Eastman, A. (1996). Enhancement of cisplatin-induced cytotoxicity by 7-hydroxystaurosporine (UCN-01), a new G2-checkpoint inhibitor. *Clinical cancer research: an official journal of the American Association for Cancer Research*, 2, 791-797.
- Bushra, R.R., and Bastwrous, A.E. (2022). Possible Protective Role of L-carnitine against Cisplatin-induced Testicular Changes in Adult Male Albino Rats: A Histological and Morphometric Study. *Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry*, 14, 81-94.
- Chabner, B.A., Amrein, P.C., Druker, B.J., Michaelson, M.D., Mitsiades, C.S., Goss, P.E., Ryan, D.P., Ramachandra, S., Richardson, P.G., and Supko, J.G. (2006). Antineoplastic agents. *The pharmacological basis of therapeutics*, 9/e, 1315-1465.
- Dada, O., and Nduka, E. (1980). Endocrine function and haemoglobinopathies: relation between the sickle cell gene and circulating plasma levels of testosterone, luteinising hormone (LH) and follicle stimulating hormone (FSH) in adult males. *Clinica Chimica Acta*, 105, 269-273.
- El Shafai, A., Zohdy, N., El Mulla, K., Hassan, M., and Morad, N. (2011). Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of ascorbic acid. *Food and chemical toxicology*, 49, 734-743.
- Essawy, S.S., Abdelfatah, A.A., and Abo-El-Matty, D.M. (2017). Pretreatment with montelukast and curcumin protect against etoposide-induced testicular toxicity in rats: role of

- oxidation, inflammation and apoptosis. *Records of Pharmaceutical and Biomedical Sciences*, 1, 52-69.
- Fischer, A.H., Jacobson, K.A., Rose, J., and Zeller, R. (2008). Hematoxylin and eosin staining of tissue and cell sections. *Cold Spring Harbor Protocols* 2008, pdb. prot4986.
- Ilbey, Y.O., Ozbek, E., Simsek, A., Otunctemur, A., Cekmen, M., and Somay, A. (2009). Potential chemoprotective effect of melatonin in cyclophosphamide- and cisplatin-induced testicular damage in rats. *Fertility and sterility*, 92, 1124-1132.
- Jiang, X., Xing, X., Zhang, Y., Zhang, C., Wu, Y., Chen, Y., Meng, R., Jia, H., Cheng, Y., and Zhang, Y. (2021). Lead exposure activates the Nrf2/Keap1 pathway, aggravates oxidative stress, and induces reproductive damage in female mice. *Ecotoxicology and environmental safety*, 207, 111231.
- Krishna, H., Changil, A., Srinivas, M., Roy, T.S., and Jacob, T.G. (2018). Ultrastructural study of rat testis following conventional phototherapy during neonatal period. *Journal of Microscopy and Ultrastructure*, 6, 205.
- Li, D., Liu, Q., Gong, Y., Huang, Y., and Han, X. (2009). Cytotoxicity and oxidative stress study in cultured rat Sertoli cells with methyl tert-butyl ether (MTBE) exposure. *Reproductive toxicology*, 27, 170-176.
- Moradi, M., Goodarzi, N., Faramarzi, A., Cheraghi, H., Hashemian, A.H., and Jalili, C. (2021). Melatonin protects rats testes against bleomycin, etoposide, and cisplatin-induced toxicity via mitigating nitro-oxidative stress and apoptosis. *Biomedicine & Pharmacotherapy*, 138, 111481.
- Osama, E., Galal, A.A., Abdalla, H., and El-Sheikh, S.M. (2019). *Chlorella vulgaris* ameliorates testicular toxicity induced by deltamethrin in male rats via modulating oxidative stress. *Andrologia*, 51, e13214.
- Priyadarshani, I., and Rath, B. (2012). Commercial and industrial applications of micro algae—A review. *Journal of Algal Biomass Utilization*, 3, 89-100.
- Ramplung, R., James, A., and Papanastassiou, V. (2004). The present and future management of malignant brain tumours: surgery, radiotherapy, chemotherapy. *Journal of Neurology, Neurosurgery & Psychiatry*, 75, ii24-ii30.
- Sato, S., Adachi, A., Sasaki, Y., and Ghazizadeh, M. (2008). Oolong tea extract as a substitute for uranyl acetate in staining of ultrathin sections. *Journal of microscopy*, 229, 17-20.
- Silici, S., Ekmekcioglu, O., Eraslan, G., and Demirtas, A. (2009). Antioxidative effect of royal jelly in cisplatin-induced testes damage. *Urology*, 74, 545-551.
- Vartak, A., Sonawane, S., Alim, H., Patel, N., Hamrouni, L., Khan, J., and Ali, A. (2022). Medicinal and Aromatic Plants in the Cosmetics Industry. In *Medicinal and Aromatic Plants of India*, Vol 1 (Springer), pp. 341-364.
- Wang, F., and Han, D. (2019). Sertoli cell phagocytosis: an essential event for spermatogenesis. *Male reproductive health*, 126-319.
- Zainul Azlan, N., Mohd Yusof, Y.A., and Makpol, S. (2020). *Chlorella vulgaris* ameliorates oxidative stress and improves the muscle regenerative capacity of young and old Sprague-Dawley rats. *Nutrients*, 12, 3752.