Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2022, Issue-3, pp-164-170
https://bfszu.journals.ekb.eg/journal
Research Paper DOI:**10.21608/bfszu.2022.104673.1097**

Ameliorative effects of Ellagic acid against toxicity of cisplatin in male rats

Hani M. Abdelsalam[1*], Abdelaziz A Diab[1], Atef G. Hussien[2], Josef A. Aziz[3] and Nourhan I. Ibrahim[1]
[1]Department of Zoology, Faculty of Science, Zagazig University, Egypt.
[2]Department of Biochemistry, Faculty of Medicine, Zagazig University, Egypt.
[3]Department of Anatomy, Faculty of Medicine, Zagazig University, Egypt.
* Corresponding Author: **hmabdelsalam@science.zuedu.eg**

ABSTRACT: Cisplatin (Cis) is a regularly used medicine to treat cancer, particularly breast cancer, however it has adverse effects that impact other bodily systems. The goal of this study is to employ Ellagic acid (Ell) to reduce the drug's side effects. A total of forty male albino rats are randomly allocated into four groups: Control, Cis, Cis+Ell, and Ell acid. Serum lipid profile and sexual hormones (FSH, LH, and testosterone) were measured biochemically, and liver slices from all groups were processed for histological analysis. Our findings demonstrated that following Cis administration, Ell treatment resulted in significant improvements in all biochemical parameters, with Ell causing considerable reductions in cholesterol, triglycerides, and LDL while increasing HDL levels. Ell induced a considerable increase in the concentrations of FSH, LH, and testosterone in the Cis+Ell group, despite Cis dramatically lowering these levels in the second group. Ell can promote a great recovery in the hepatic architecture following a Cis-induced deformation, according to histopathological analysis. Finally, Ell therapy can help to mitigate the harmful effects of Cis delivery.

KEYWORDS:* *Cisplatin - Ellagic acid – Lipid profile – Hormonal assay- Hepatocytes

---

Date of Submission: 06-11-2021 Date of acceptance: 27-05-2022

I. INTRODUCTION

Cisplatin (Cis) is a cytotoxic drug used to treat solid tumors such as those of the ovary, bladder, and testicles [1, 2]. Its clinical usage in oncology has been limited by its documented nephrotoxicity, hepatotoxicity, and neurotoxicity [3, 4]. Hepatotoxicity at high doses is caused by the accumulation of cisplatin in liver cells, which causes inflammation, an increase in free radical generation, and a decrease in antioxidant enzymes [5, 6]. Ellagic acid (Ell) is a phenolic component in green tea, walnuts, and fruits such as grapes, berries, and pomegranates. It has wide range of biological properties [7]. including antioxidant [8, 9] and anti-inflammatory [10, 11] activities. Researchers discovered that ELL reduces cisplatin-induced lipid peroxidation and increases GPx and CAT levels in rat livers. The ELL protects against the negative effects of -OH and O2- [12].

II. MATERIAL AND METHOD

Chemicals:
Cisplatin (250mg, trace metal basis, code193762500) was obtained from Acros organics the USA.
Ellagic acid, 97% (2.5GR, code 117740025) was also obtained from Acros organics USA.
Animals and experiment design:
In this study, 40 healthy male adult albino rats weighing 150–180 g were used. The animals were obtained from Theodor Bilharz Research Institute, Cairo, Egypt and kept under normal conditions with free access to food and water. All animals were housed in hygienic plastic cages in well-ventilated rooms with exhaust fans; they were

fed a standard pellet diet and had free access to water daily. All animal procedures were carried out with the approval of the Ethics Committee of the National Research Center, Egypt, and following recommendations of the institutional animal care and use committee of the Zagazig University (ZU-IACUC/3/f/21/2021).
The rats were randomly divided into four groups, each group containing 10 rats as follow;

1-Control group.
2-Cisplatin group.
3- Ellagic acid group.
4- Cisplatin+Ellagic acid group

Cisplatin was administered intraperitoneally (IP) to animals in a single dose of 7 mg/kg (isotonic saline was the vehicle for administering cisplatin).
Ellagic acid was dissolved in maize oil at a concentration of 5 mg/ml and given to animals by gavage at a dose of 10 mg/kg/day.
(Cisplatin and ellagic acid doses and dosing times were chosen based on previous research.)[13].
1-The first group of rats received isotonic saline and served as the control group.
2-The second group of rats (cisplatin group) received a single intraperitoneal injection of cisplatin (7 mg/kg).
3-The rats in the third group (Ellagic acid group) were given ellagic acid (10 mg/kg/day) for ten days.
4-After a single cisplatin IP injection, the fourth group of rats (Cisplatin + Ellagic acid group) was given ellagic acid for 10 days.

Sample collection:

The rats were killed at the end of 10 days using ether anesthesia. The liver samples were taken out and kept away from the light. The liver samples were cleaned three times in cold isotonic saline (0.9 percent v/w) and kept at -20 °C until they were analysed.
Blood was collected in test tubes from this dislocation, which was then centrifuged to remove serum, which was maintained in an Eppendorf tube in a deep freezer at -20 °C.

Determination of lipid profile:

Determined calorimetrically according to methods of Artiss and Zack [14].

Hormonal assay:

1-Determination of FSH:

The method for the quantitative determination of FSH is a sandwich chemiluminescence immunoassay according to methods of McCann et al [15].

2-Determination of LH:

The method for the quantitative determination of LH is a sandwich chemiluminescence immunoassay according to methods of McCann et al [15].

3-Determination of Testosterone:

The LIAISON® Testosterone assay's method for quantitative determination of testosterone is a direct, competitive, chemiluminescence immunoassay (CLIA) according to methods of Klee and Heser **[16].**

Histopathological studies:

Liver specimens were fixed in in 10% formalin, dehydrated in alcohol in ascending grades (70%, 80%, 90%, 95%, and 100%) of ethanol, cleared in xylene, and then embedded in paraffin wax blocks. Serial sections of 4-6 µm thickness were stained with: Hematoxylin and Eosin stain [17] for basic histological analysis.

III. RESULTS

Table (1): Serum lipid profile in various studied groups

Parameter	Control	Cis	Ell + Cis	Ell
Cholesterol	110.1 ± 7.967	165.2 ± 15.60 a	133.5 ± 7.271 a,b	105.2 ± 6.656
TG	96.81 ± 6.851	150.2 ± 8.241 a	119.9 ± 7.017 a,b	93.07 ± 6.526
HDL	63.92 ± 3.941	38.27 ± 2.679 a	49.37 ± 3.916 a,b	62.49 ± 4.324
LDL	26.79 ± 5.538	98.02 ± 10.52 a	60.67 ± 3.408 a,b	23.68 ± 6.941

All values are expressed as mean ±SD, *n*=10. **(a)** significant vs control group, **(b)** significant vs Cis. A one-way ANOVA followed by post hoc Tukey test multiple comparisons between groups.
Cis-treated group exhibited statistically significantly higher mean total cholesterol, triglycerides, LDL compared to control group (P<.05). Ell + Cis group revealed significant lower mean total cholesterol, triglycerides, LDL

levels compared to Cis group (P>.05). Moreover, statistically significant increase of HDL was evident in Ell + Cis group compared to Cis group (P<.05). Statistical results of various studied groups are presented in **Table 1.**

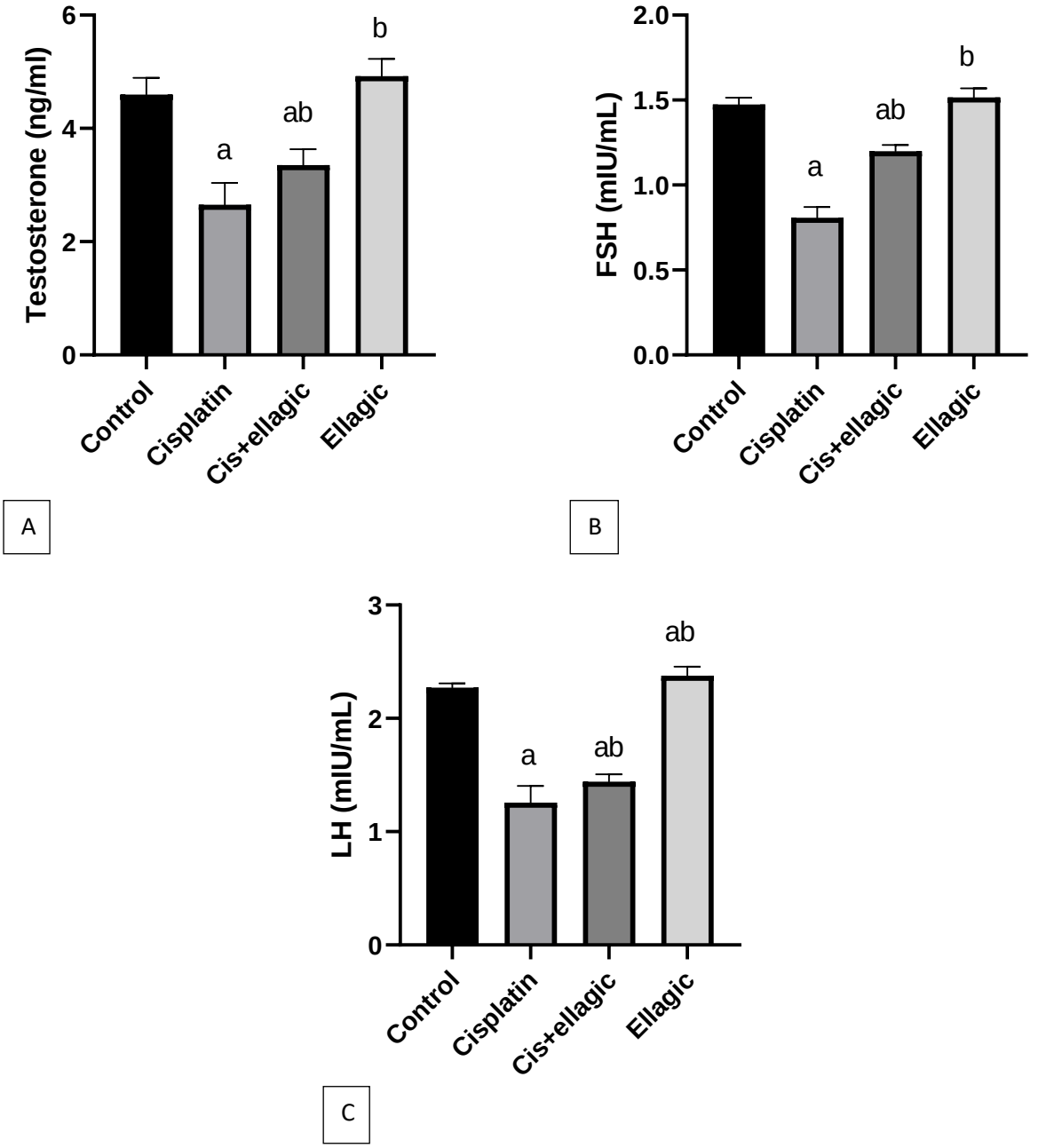

Fig 1: Testosterone (A), FSH (B) and LH (C) levels in in various studied groups All values are expressed as mean ±SD, *n*=10. **(a)** significant vs control group, (**b)** significant vs Cis. A one-way ANOVA followed by post hoc Tukey test multiple comparisons between groups.

Cis-treated group exhibited statistically significantly lower mean Testosterone, FSH and LH compared to control group (P<.05). Ell + Cis group revealed significant higher mean Testosterone, FSH and LH compared to Cis group (P>.05). Statistical results of various studied groups are presented in Fig 1.

Histopathological Results:

Normal hepatic lobules with branching cords of hepatocytes extending from the central vein were seen in the control (Fig. 2 a, 2b) and ellagic acid (Fig. 2 c, 2d) groups. Hepatocytes were organised around the sinusoids of the liver. The portal triad is formed by branches of the portal vein, bile duct, and hepatic artery at the periphery of each lobule. Hepatocytes were polyhedral cells with a rounded nucleus. Two nuclei were seen in certain hepatocytes.

There was dilated congested central vein, centrilobular necrosis, and bile duct proliferation in the cisplatin group. Hepatocytes with pyknotic nuclei and hydropic degeneration were also seen (fig.2 e, f). These alterations were reversed in rats given cisplatin and ellagic acid (Fig.2 g,2 h).

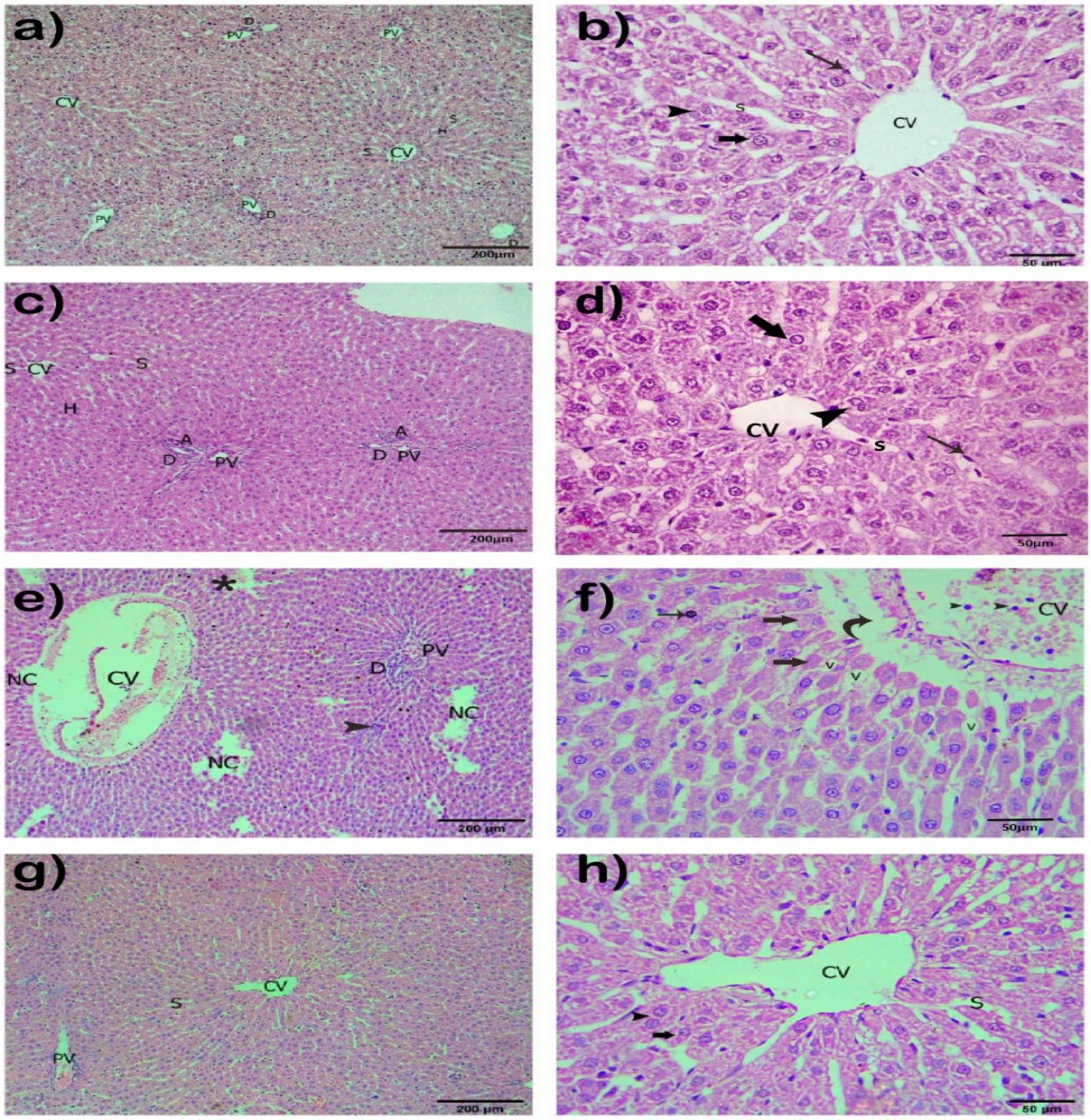

Figure (2) photomicrographs of sections in the liver of all groups: (**a,c)** Control and ellagic groups show hepatic lobule with central vein (CV), cords of hepatocytes (H) separated by narrow sinusoid (s) Branches of portal vein (PV), bile duct (D) at the periphery. At higher magnification **(b, d)**, hepatocytes are polygonal with single rounded vesicular nucleus (arrowhead), sometimes with double nuclei (thick arrow). Kupffer cells (thin arrow) are visible in the wall of hepatic sinusoids (s); Cisplatin group **(e)** shows dilated congested central vein (CV) and sinusoids (asterisk) with centrilobular necrosis (NC) and cellular infiltrates (arrowheads). Proliferation of bile ducts (D) and congested portal vein (PV) can be seen in the portal area. At higher magnification **(f)** part of markedly dilated and congested central vein containing inflammatory cells (arrowheads) with separation of its wall (curved arrow). Vacuolation (v), apoptotic hepatocytes (thick arrow) and hepatocytes with pyknotic nuclei (thin arrow) can be detected; **(g, h)** Ellagic+ cisplatin group nearly normal hepatic histology except for congested portal vein (PV) and slightly dilated central vein.

(H&E (a, c, e, g): X 100; (b, d, f, h): X400)

IV. Discussion

The liver plays a major role in controlling plasma levels of cholesterol, thus when there are drug-induced liver impairments, serum total cholesterol (TC) and LDL-cholesterol levels will be raised [18]. Our findings demonstrated that Cis treatment resulted in a substantial increase in total cholesterol, triglycerides, and LDL levels when compared to the normal levels of the control group, whereas Ell treatment resulted in a significant drop in these parameters. The significant increase in serum total cholesterol (TC), triglycerides (TG), and LDL-cholesterol after rats were exposed to cisplatin could be attributed to the drug's adverse effect, which results in hepatocellular dysfunction and impaired lipid metabolism, which are in consistent with the findings of Akindele et al [19]. It has been established that high TC, LDL-C, and low HDL-C levels are risk factors for cardiovascular disease [20]. Also, these results in line with Elhemely,etal [21]. who stated that pretreatment with ellagic acid improved the lipid profile significantly. Similarly to ellagic acid, the hypocholesterolemic effect is mostly mediated by inhibition of HMG-CoA reductase [22].

Cis is a highly potent chemotherapeutic drug, but its usage is limited primarily due to two factors: Cis resistance and severe toxicity to normal tissues, particularly nephrotoxicity, neurotoxicity, and testicular injury [23]. The results of this study revealed that Cis-treated men had statistically lower mean Testosterone, FSH, and LH levels than the control group. In comparison to the Cis group, the Ell + Cis group had significantly higher mean Testosterone, FSH, and LH levels. Physiological and pathohistological changes resulting from oxidative stress and DNA damage are among the mechanisms underlying Cis-induced testicular injury [24].

Spermatogenesis is regulated mainly by endocrine factors such as FSH and LH, and by paracrine factors such as testosterone. Reduced amounts of those regulators may result in the spermatogenetic process failing [25]. Cis-treated rats demonstrated a drop in testosterone levels, which was consistent with earlier research [26, 27]. This reduction in the hormonal level may be explained by two theories, that Cis induced severe damages on Leydig and Sertoli cells resulting from the increased generation of ROS [28] , or it may occur through interference with LH receptor expression and FSH [29].

according to our findings EA administration definitely alters sperm parameters and total sexual hormone function, and can also increase the spermatogenesis rate in animals with damaged sperm. The powerful free radical scavenging properties of EA as a new antioxidant may be responsible for the recovery of testosterone, FSH, and LH levels, as well as germinal cell count, following EA treatment [30].

The findings of this investigation demonstrated Ell acid's potential to reverse the significant harm caused by Cis. Cisplatin damages the liver by causing oxidative damage to hepatocyte lipids and DNA [31]. Cisplatin caused centrilobular necrosis in the present investigation, as well as hydropic degeneration of liver cells, congestion, and dilatation of the central and sinusoids, confirming the findings of Umar Ijaz and Ashraf [32]. and Elkomy, Abdelhiee [33]. In agreement with Alrashed and El-Kordy [34]. Enlargement of portal tracts with dilatation of branches of portal veins and proliferation of bile ductules was observed. In this study treatment with 10 mg/kg of Ell for 10 days ameliorated the toxicity of cisplatin on the liver. In the study of Goyal, Koul [35], Ell (10 mg/kg for 6 weeks) administration Significantly reduced the damage produced by 5 mg/kg Cis given once a week for 4 weeks and improved the histological changes detected, implying that Ell administration could be a possible chemopreventive candidate.

Abbreviations: Cis: cisplatin**, Ell:** Ellagic acid**, LDL:** low density lipoprotein**, FSH:** Follicle-stimulating hormone and **LH:** luteinizing hormone

Acknowledgements: We grateful to those who instilled in us the value of education and the rewards and opportunities it can generate to our parents, who supplied us with enthusiasm, support, and creative insight.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding source

This study was not funded by any source.

REFERENCES

1. Dasari, S. and P.B. Tchounwou, *Cisplatin in cancer therapy: molecular mechanisms of action.* European journal of pharmacology, 2014. **740**: p. 364-378.
2. Cui, W., et al., *Cisplatin-induced response of c-jun N-terminal kinase 1 and extracellular signal-regulated protein kinases 1 and 2 in a series of cisplatin-resistant ovarian carcinoma cell lines.* 2000. **29**(4): p. 219-228.
3. Barabas, K., et al., *Cisplatin: a review of toxicities and therapeutic applications.* 2008. **6**(1): p. 1-18.
4. Zicca, A., et al., *Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats.* 2002. **442**(3): p. 265-272.

5. de Oliveira Mora, L., et al., *The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats.* 2003. **47**(6): p. 517-522.
6. Kim, Y.K., et al., *Effects of antioxidants and Ca2+ in cisplatin-induced cell injury in rabbit renal cortical slices.* 1997. **146**(2): p. 261-269.
7. Vattem, D. and K.J.J.o.f.b. Shetty, *Biological functionality of ellagic acid: a review.* 2005. **29**(3): p. 234-266.
8. Baek, B., et al., *Ellagic acid plays a protective role against UV-B-induced oxidative stress by up-regulating antioxidant components in human dermal fibroblasts.* 2016. **20**(3): p. 269.
9. Han, D.H., M.J. Lee, and J.H.J.A.r. Kim, *Antioxidant and apoptosis-inducing activities of ellagic acid.* 2006. **26**(5A): p. 3601-3606.
10. Rogerio, A.P., et al., *Anti-inflammatory effects of Lafoensia pacari and ellagic acid in a murine model of asthma.* 2008. **580**(1-2): p. 262-270.
11. Cornélio Favarin, D., et al., *Anti-inflammatory effects of ellagic acid on acute lung injury induced by acid in mice.* 2013. **2013**.
12. Priyadarsini, K.I., et al., *Free radical studies of ellagic acid, a natural phenolic antioxidant.* 2002. **50**(7): p. 2200-2206.
13. Türk, G., et al., *Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid.* 2008. **89**(5): p. 1474-1481.
14. Artiss, J.D. and B.J.H.o.l.t. Zak, *Measurement of cholesterol concentration.* 1997. **2**: p. 189-205.
15. McCann, S.M., et al., *Hypothalamic control of FSH and LH by FSH-RF, LHRH, cytokines, leptin and nitric oxide.* 1998. **5**(3-4): p. 193-202.
16. Klee, G.G. and D.W. Heser. *Techniques to measure testosterone in the elderly*. in *Mayo Clinic Proceedings*. 2000. Elsevier.
17. Kiernan, J.A.J.S., *Histological and histochemical methods: theory and practice.* 1999. **12**(6): p. 479.
18. Atawodi, S.E.-O., et al., *Effect of methanolic extract of Tetrapleura tetraptera (Schum and Thonn) Taub leaves on hyperglycemia and indices of diabetic complications in alloxan–induced diabetic rats.* 2014. **4**(4): p. 272-278.
19. Akindele, A.J., et al., *Ameliorative effect of hydroethanolic leaf extract of Byrsocarpus coccineus in alcohol-and sucrose-induced hypertension in rats.* 2014. **4**(3): p. 177-188.
20. Kataraki, P. and K.V.M.V.S. Varma, *Study of Serum Lipid Profile in Individuals Residing and Around Nalgonda.* 2012.
21. Elhemely, M.A., et al., *Rosuvastatin and ellagic acid protect against isoproterenol-induced myocardial infarction in hyperlipidemic rats.* 2014. **3**(4): p. 239-246.
22. Kannan, M.M. and S.D.J.M. Quine, *Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidaemia during myocardial infarction in rats.* 2013. **62**(1): p. 52-61.
23. Abdel Moneim, A.E., M.S. Othman, and A.M.J.B.r.i. Aref, *Azadirachta indica attenuates cisplatin-induced nephrotoxicity and oxidative stress.* 2014. **2014**.
24. Fallahzadeh, A.R., et al., *Evaluation of the effect of pentoxifylline on cisplatin-induced testicular toxicity in rats.* 2017. **33**(3): p. 255-263.
25. Erkekoglu, P., et al., *The effects of di (2-ethylhexyl) phthalate exposure and selenium nutrition on sertoli cell vimentin structure and germ-cell apoptosis in rat testis.* 2012. **62**(3): p. 539-547.
26. Ahmed, M.M., et al., *L-carnitine protects against testicular dysfunction caused by gamma irradiation in mice.* 2014. **116**(6): p. 1046-1055.
27. Aziz, R.L.A., et al., *Dose-dependent ameliorative effects of quercetin and l-Carnitine against atrazine-induced reproductive toxicity in adult male Albino rats.* 2018. **102**: p. 855-864.
28. Tousson, E., et al., *Abrogation by curcumin on testicular toxicity induced by cisplatin in rats.* 2014. **2**(3): p. 64-8.
29. Elshiekh, A.A., et al., *Possible Protective Effect of Ginger Extract and Beetroot Juice Against Cisplatin Induced Testicular and Cytogenetic Toxicity in Adult Male Albino Rats.* 2019. **76**(5): p. 4046-4054.

30. Olfati, A., H.J.J.o.E.B. Khamisabadi, and Physiology, *Ellagic Acid Improves Testicular Dysfunction via Autophagy in a Tamoxifen-Injured Rat Model.* 2020. **56**(3): p. 265-276.
31. Casares, C., et al., *Reactive oxygen species in apoptosis induced by cisplatin: review of physiopathological mechanisms in animal models.* 2012. **269**(12): p. 2455-2459.
32. Ijaz, M.U., et al., *Remedial effects of casticin as an antioxidant on cisplatin induced oxidative damage in rat liver.* 2020. **32**(1): p. 1100-1105.
33. Elkomy, A., et al., *L-Carnitine Mitigates Oxidative Stress and Disorganization of Cytoskeleton Intermediate Filaments in Cisplatin-Induced Hepato-Renal Toxicity in Rats.* Frontiers in Pharmacology, 2020. **11**.
34. Alrashed, A.A. and E.A. El-Kordy, *Possible protective role of panax ginseng on cisplatin-induced hepatotoxicity in adult male albino rats (Biochemical and Histological Study).* Journal of microscopy and ultrastructure, 2019. **7**(2): p. 84.
35. Goyal, Y., A. Koul, and P. Ranawat, *Ellagic acid ameliorates cisplatin induced hepatotoxicity in colon carcinogenesis.* Environmental toxicology, 2019. **34**(7): p. 804-813.