pp. 66-80 https://dx.doi.org/10.4314/johasam.v7i2.6

Restorative effect of *Annona Muricata*extract on Histomorphology of Testicular Tissue of Cadmium Tumor Induced Albino Rats

Ehiremen, S.E[1]., Iyare, G.I[1].,Omorodion, N.T[2]
Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo State University, Uzairue[1]
Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medicine University of Benin, Benin city, Nigeria[2]
Department of Anatomy, Faculty of Basic Medical Science, Olabisis Onabanjo University, Ogun State, Nigeria[3]
Correspondence: OmorodionNosa Terry / 08136742270 / Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria/E-mail - terry.omorodion@uniben.edu/Orcid ID: 0000-0002-5500-5293

Abstract

Cadmium is a harmful heavy metal that is widely distributed in the environment. This study aimed to investigate the effects of *Annona muricata* seed extract on cadmium-induced testicular toxicity in adult male Wistar rats. A total of 35 adult male Wistar rats were randomly divided into five groups (A–E)) of 7 rats each. Group A (the control group) received distilled water only; group B-E received 2 mg/kg of cadmium (S.C.) and 100 mg/kg body weight of *Annona muricata*, 2 mg/kg of cadmium (S.C.) and 200 mg/kg body weight of *Annona muricata*, and 2 mg/kg of cadmium and 300 mg/kg body weight of *Annona muricata* respectively. After 17 days of treatment, the animals were brought out, weighed, and sacrificed via cranial dislocation. Testicular, seminal fluid, and blood samples were taken for examination. SPSS version 21 was used in the analysis. The results of the study showed that the *Annona muricata* aqueous seed extract increased the levels of superoxide dismutase (SOD) and catalase (CAT) in a dose-dependent manner compared to the cadmium-only group. The relative weight of the testis increased significantly in the treated groups compared to the cadmium-only group. Testosterone levels also increased significantly ($P < 0.05$) in the treated groups compared to the group A (control). The extract showed a significant protective effect against cadmium-induced toxicity, as indicated by the higher testosterone levels in treated groups compared to the untreated group. Medicinal plants like *Annona muricata* can be used to treat heavy metal toxicity in traditional medicine.

Introduction

Soursop, or graviola, scientifically known as *Annona muricata*, is a tropical evergreen fruit tree that belongs to the *Annonaceae* family and is distributed in many tropical and subtropical regions worldwide (Rady *et al.*, 2018; Patel and Patel, 2016; Moghadamtousi et al., 2015).Local communities in tropical Africa and South America have been using different parts of the plant, including the bark, fruits, leaves, pericarp, seeds, and roots, in traditional medicine to treat various ailments, including cancer and tumours (Blancke, 2016). Experimental studies have shown that different parts of *Annona muricata* have anticarcinogenic and genotoxic effects (Yajid et al., 2018; Rady et al., 2018), cytotoxicity, and antileishmanial, antiviral, wound-healing, and antimicrobial activity (Brgido et al., 2020; Le Donne et al., 2017; Moghadamtousiesh et al., 2016).

Plant leaves contain small amounts of secondary metabolites such as tannins, steroids, cardiac glycosides, and others, according to phytochemical studies (Vijayameena et al., 2013).

Cadmium is a metal that belongs to group II B and has an atomic weight of 112.41. Cadmium is known to form ionic compounds with oxygen, chlorine, or sulphur, such as cadmium oxide, cadmium chloride, and cadmium sulphate (Rahman, 2017; Sardar, 2013). Cadmium is widely used in various industrial processes, including electroplating, galvanization, pigment production, battery manufacturing, plastic production, metal container production, and fertilizer manufacturing. It is also a by-product of zinc, lead, and copper metallurgy (Meshram & Pandey, 2019).

The male reproductive system comprises both internal (testes, epididymis, vas deferens, and prostate) and external (scrotum and penis). These structures are vital for the production, storage, and ejaculation of sperm as well as androgen synthesis (Tiwana and Leslie, 2021). Recent research has shown a decline in human sperm quality worldwide, and it is suggested that factors such as environmental exposure, dietary habits, occupational factors, and lifestyle practice may contribute to this trend (Sharma, 2013; Jurewicz, 2014; Sengupta, 2017). Disorders of the male reproductive system are typically caused by low testosterone levels, which can lead to various symptoms such as decreased libido, infertility, and muscle loss (Lee & Tillman, 2016). Approximately 7% of men are affected by infertility, which can result from factors acting at different levels of the reproductive system (Dimitriadis, 2017).

Exposure to toxic metals can lead to the development of reactive oxygen species, which contribute to the formation of malignant tumours and cancer. Current treatments for cancer, such as chemotherapy and radiotherapy, can be harmful to patients, leading to a focus on alternative therapies. Herbal medicines have been used for centuries and are still commonly used in many countries as a primary source of medical treatment due to their natural antiseptic properties. Researchers are exploring the potential use of terrestrial plant extracts to prepare nanomaterial-based drugs for various metabolic diseases, including cancer (Greenwell & Rahman, 2015; Gezici & Ekerolu, 2019). This study will evaluate the protective effect of the aqueous seed extract of *Annona muricata* against cadmium-induced testicular malignant tumours in adult male Wistar rats.

The proposed study aims to evaluate the histomorphologica and biochemical restorative effect of *Annona muricata* extract. Histomorphology is the study of the microscopic structure of tissues and cells. The proposed study will examine the changes in testicular tissue structure in response to cadmium exposure and treatment with *Annona muricata* extract. This is important because histomorphological and biochemical changes can provide insight into the mechanism of action of the extract and its potential therapeutic effects. The study is justified due to the need for an effective and safe treatment for cadmium-induced testicular tumours.

Materials and methods

Reagents and materials

Cadmium chloride, NaCl, phosphate buffer, Epinephrine, Carbonate buffer, phosphate buffer, glacial acetic acid, Dichromate/acetic acid solution, Hydrogen peroxide, distilled water, potassium hepaxodichromate, hematoxylin and eosin, paraffin, xylene, alcohol, formal saline, animal cages, mug cup, mug plate, weighing scale, syringe (100ul, 2 ml, 5 ml), oral cannula (18g), dissecting set, dissecting board, universal sample bottle (5 ml and 20 ml), Rotatory microtome, Water bath, Wooden block, glass slide, scissors,

Animal management

Male Wistar albino rats were obtained from the animal house, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ogun State, Nigeria. This study was in accordance with established guidelines for the care and use of laboratory animals in biomedical

research and teachings approved by the Institute of Laboratory Animal Resources, National Research Council (2010).

Animal grouping and care

The rats were housed in plastic cages, kept under a 12-hour light/12-hour dark cycle, and allowed free access to feed and water throughout the experimental period. Thirty-five (35) adults healthy male Wistar rats weighing between 150 and 250g were used for this experiment. The rats were randomly grouped into seven groups after a period of fourteen days of acclimatization, as shown in Table 3.1 below.

Table 3.1: animal grouping

Group	Treatments	Number of rats per group
A(control group)	Distilled water only	7
B	2 mg/kg of cadmium (S.C)	7
C	2 mg/kg of cadmium (S.C) and 100 mg/kg body weight of *Annona muricata* (P.O)	7
D	2 mg/kg of cadmium (S.C) and 200 mg/kg body weight of *Annona muricata* (P.O)	7
E	2 mg / kg of cadmium (S.C) and 300 mg/kg body weight of *Annona muricata* (P.O)	7

S.C: subcutaneous, P.O: oral route of administration

Preparation of the aqueous seed extract of *Annona muricata*

*Annona muricata*fruit was purchased from the Ikenne market in the Ikenne local government area of Ogun state, Nigeria. The fruit was peeled to expose the seed. *Annona muricata* seed was washed with clean water and air-dried at room temperature. The seed was then milled into powder, and 500g of the powder was macerated in one litre of distilled water for 24 hours. The mixture was then filtered, and the filtrate was concentrated using a rotary evaporator at 60 °C. The concentrated filtrate was collected in a bottle and kept at room temperature before use. The concentrate was reconstituted in distilled water to prepare 100 mg/kg, 200 mg/kg, and 300 mg/kg of *Annona muricata* used in this study.

Preparation of cadmium

One milligramme of Cadmium chloride was suspended in 5 ml of 0.9% Nacl.

Administration of treatment

The administration of cadmium was done according to the method described by Gaurav et al. (2010), with a little modification. The rats were exposed to cadmium for 2 days, and *Annona muricata* aqueous seed extract was administered concomitantly for 15 consecutive days.

Acute Toxicity Studies

An acute toxicity study was carried out to determine the LD50 of the aqueous seed extract of *Annona muricata*. This was done in two phases, according to the study of Sherif, Baba, and Abdullahi (2017).

Animal sacrifice and determination of organ weight

The animals were sacrificed by cervical dislocation six hours after the expiration of the research. The testis was excised following a midline abdominal incision and carefully brought out; the organ weight was determined per 100 gramme body weight using a weighing scale (kerroBL20001).

Procedure for blood collection
Blood was collected from the orbital venous sinus; the rat was restrained, the neck gently scuffed, and the eye made to bulge. A capillary tube was inserted dorsally into the eye, and blood was allowed to flow by capillary action through the capillary tube into a sample bottle.

Studies on oxidative stress
The study determined the effect of various doses of *Annona muricata* on oxidative stress in the testis of a cadmium-induced malignant tumour.

Determination of superoxide dismutase (SOD) activity
SOD activity in the testis of the rats was determined by the method of Misra and Fridovich (1972).

Determination of catalase activity
Tissue catalase activity was determined according to the method of Sinha (1972).

Spectrophotometric determination of H_2O_2
Determination of testosterone levels
Sample tubes that contained blood samples were gathered and placed in an incubator at 37°C for 30 minutes. After coagulation, the tubes were placed into the centrifuge at 5000 rpm. Sera was pipetted and transferred into new, labelled tubes sealed with paraffin, and testosterone was measured using the radioimmunoassay method (RIA).

Sperm analysis
Sperm collection
Sperm was collected from the epididymis using the diffusion method as described by Seed et al. (1996).

Determination and classification of sperm Morphology
This was done as described by Saalu, Akunna, and Ajayi (2013). In this study, a spermatozoon was considered morphologically abnormal if it had a rudimentary tail, a round or detached head, and was expressed as a percentage of morphologically normal sperm as described by Adelakun et al. (2018), Ibeh and Omorodion (2018).

Determination of sperm count and motility
Spermatozoa in the epididymis were counted by a modified method described by Saalu *et al*. (2013).

Haematoxylin and eosin staining method and protocol
Protocol described by Omorodion et al. (2021)

Masson trichrome staining methods
Standard Protocols was adopted

Photomicrography

Image acquisition and analysis: A bright-light microscope (10–40x magnification objective) was used. Digital camera: OMAX Toup View 3.7 is attached to the PC; HP is used. Java Application Software (image J Software) is used

Statistical analysis

The descriptive statistics of mean, standard deviation, and inferential statistics were used for this study. The data was subjected to statistical testing and analysis with the aid of Statistical Packages for Social Sciences (SPSS) version 21 and Microsoft Excel 2021 for Windows using the T-test method of data analysis. 0.05 was the alpha level of significance (p 0.05*).*

Results

Effect of the oral administration of the aqueous seed extract of *Annona muricata* against oxidative stress induced by Cadmium in the testis of adult male Wistar rats

The table shows the effect of oral administration of *Annona muricata* extract on oxidative stress induced by cadmium in the testis of male Wistar rats. Group A was given distilled water only; Group B received 2 mg/kg of cadmium subcutaneously; Group C received 2 mg/kg of cadmium and 100 mg/kg of *Annona muricata* extract orally; Group D received 2 mg/kg of cadmium and 200 mg/kg of *Annona muricata* extract orally; and Group E received 2 mg/kg of cadmium and 300 mg/kg of *Annona muricata* extract orally. Our results showed that the extract significantly increased the levels of superoxide dismutase (SOD) and catalase (CAT) in a dose-dependent manner compared to the cadmium-only group (Group B). The values were significantly different between the groups, with increasing significance at higher doses of the extract.

Table 2: Effect of the oral administration of the aqueous seed extract of *Annona muricata* against oxidative stress induced by Cadmium in the testis of adult male wistar rats

Groups	A	B	C	D	E
Treatment	Distilled	2mg/kg of Cadmium	2 mg/kg of cadium (s.c) and 100mg/ kg b/w of *A, muricata*	2mg/kg of cadium and 200m / kg b/w of *A, muricata*	2mg/kg of cadium and 300mg / kg b/ w of *A, muricata*
SOD (µmol/ml/ min/mg/pro)	30±5.34	14.16±0.89	[A]17.24±2.01 [A, B]	28.21±2.31 [B, C]	30.68±3.98 [B, C]
CAT (µmol/ml/ min/mg/pro)	30.29±0.85	8.09±4.09	[A]13.97±4.13 [A]	22.94±3.83 [A, B, C]	23.02±6.89 [B]

Each value is an expression of mean ± SEM. (P <0.05)

[A] - Values were significant when compared to group A, [B]-Values were significant when compared to group B, [C]- Values were significant when compared to group C, [D] Values were significant when compared to group D

The table shows the effect of the oral administration of *Annona muricata* on the relative weight of the testis in Cadmium-induced toxicity in adult male Wistar rats. The table shows that the relative weight of the testis significantly decreased in Group B, while there was a significant increase in Groups C, D, and E when compared to Group B. Group A showed no significant changes.

Table 3: Effect of the oral administration of the aqueous seed extract of *Annona muricata* on the relative weight of the testis in Cadmium induced toxicity in the testis of adult male wistar rats.

Groups	A	B	C	D	E
Treatment	Distilled	2mg/kg of Cadmium	2 mg/kg of cadium (s.c) and 100mg/ kg b/w of *A, muricata*	2mg/kg of cadium and 200m / kg b/w of *A, muricata*	2mg/kg of cadium and 300mg / kg b/ w of*A, muricata*
Relative Weight of Testis (g)	1.12	1.83 ^{A}	1.43 $^{A, B}$	1.03$^{B, C}$	1.03 ^{C}

Each value is an expression of mean ± SEM. ($P < 0.05$)
^{A} - Values were significant when compared to group A, ^{B}-Values were significant when compared to group B, ^{C}- Values were significant when compared to group C, ^{D} Values were significant when compared to group D
The table shows the effect of administering the aqueous seed extract of *Annona muricata* on testosterone levels in male rats with cadmium-induced toxicity. A significant increase in testosterone levels was observed in groups treated with the extract compared to the control group (group A). The extract also showed a significant protective effect against cadmium-induced toxicity, as indicated by the higher testosterone levels in treated groups (B and C) compared to the untreated group (group D).

Table 4: Effect of the oral administration of the aqueous seed extract of Annona muricata on the testosterone level in Cadmium induced toxicity in the testis of adult male wistar rats.

Groups	A	B	C	D	E
Treatment	Distilled	2mg/kg of Cadmium	2 mg/kg of cadium (s.c) and 100mg/ kg b/w of *A, muricata*	2mg/kg of cadium and 200m / kg b/w of *A, muricata*	2mg/kg of cadium and 300mg / kg b/ w of*A, muricata*
Testosterone (Ng/ml)	2.4	0.96^{A}	1.17 ^{A}	1.7 $^{B, C}$	1.8 $^{B, C}$

Each value is an expression of mean ± SEM. ($P < 0.05$)
^{A} - Values were significant when compared to group A, ^{B}-Values were significant when compared to group B, ^{C}- Values were significant when compared to group C, ^{D} Values were significant when compared to group D

The table shows the effect of administering different doses of *Annona muricata* extract on sperm analysis in male rats with cadmium-induced toxicity. The groups were categorised based on the treatment they received. The different parameters measured include normal morphology, abnormal morphology, viscosity, motility, and dead and alive sperm counts. The values are expressed as mean ± SEM, and the significance level is indicated by letters. A significant decrease in sperm parameters was observed in groups treated with cadmium only (group B) compared to the control group (group A). However, treatment with the extract resulted in a significant improvement in sperm parameters, with the highest dose of the extract (group E) showing the most significant improvement. Overall, the results suggest that *Annona muricata* extract may have a protective effect against cadmium-induced damage to sperm parameters in male rats.

Table 5: Effect of the oral administration of the aqueous seed extract of *Annona muricata* on sperm analysis in Cadmium induced toxicity in the testis of adult male wistar rats

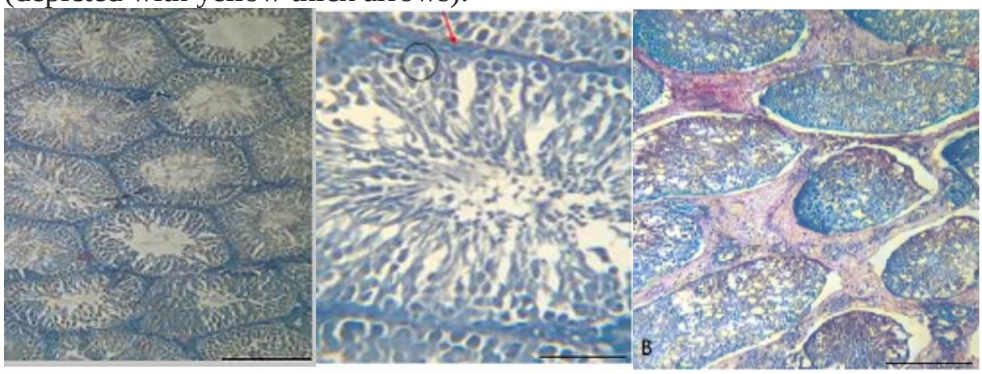
Groups	A	B	C	D	E
Treatment	Distilled	2mg/kg of Cadmium	2 mg/kg of cadium (s.c) and 100mg/ kg b/w of *A, muricata*	2mg/kg of cadium and 200m / kg b/w of *A, muricata*	2mg/kg of cadium and 300mg / kg b/ w of*A, muricata*
Normal Morphology (%)	70±0	40.5±0.01 ^{A}	60±0 $^{A, B}$	55±7.07 ^{A}	62.5±3.53 $^{A, B}$
Abnormal Morophology (%)	30±0	55±7.07 ^{A}	40±0 $^{A, B}$	33±7.07 ^{B}	37.5±3.54 $^{A, B}$
Viscosity (%)	85±7.07	45±0.07 ^{A}	60±0.01 ^{A}	65±7.07	75±7.07
Non visco sity (%)	15±7.07	70±14.14 A	40±0 $^{A, B}$	35±7.07 ^{B}	25±70.7 ^{B,C}
motile (%)	85±7.07	35±21.21 ^{A}	60±0 ^{A}	65±7.07	67.5±3.53 $^{A, C}$
Non Motile (%)	15±7.07	65±21.21	40±0 ^{A}	35±7.07	32.5±3.53 $^{A, C}$
Dead (%)	15±7.07	70±28.28 ^{A}	35±7.07	35±7.07	25±7.07
Alive (%)	85±7.07	30±25.24	65±7.07	65±7.07	75±7.07
sperm count (x 10^6 m/L)	161.5±3.54	127±7.07 ^{A}	154.5±2.12 ^{B}	15.8±2.83 ^{B}	161.3±4.94 ^{B}

Each value is an expression of mean ± SEM. ($P < 0.05$)

^{A} - Values were significant when compared to group A, ^{B}-Values were significant when compared to group B, ^{C}- Values were significant when compared to group C, ^{D} Values were significant when compared to group D, (magnification 200x) and (magnification 400x) display the testis histoarchitecture of rats exposed to cadmium chloride toxicity and the protective effect of *Annona muricata* using Masson's trichrome staining. The control group demonstrates a normal distribution of collagen fibres in the interstitial layer (indicated by a thin red arrow) between the seminiferous tubules. In the group induced with cadmium chloride (CdCl2), the tubular basement membrane thickens, and the seminiferous tubules undergo degeneration and atrophy. There is an excessive amount of collagen fibres present in the interstitial layer (red thin arrow), along with the presence of multiple spermatid giant cells (highlighted by a red

circle). When induced with cadmium chloride and treated with a 100 mg/kg aqueous seed extract, the seminiferous tubules show mild regeneration, with a thickened distribution of collagen fibres in the interstitial layer (indicated by a thin black arrow). Treatment with a 200 mg/kg aqueous seed extract results in a decrease in collagen fibre deposition (yellow thin arrow) compared to the normal control. Lastly, treatment with a 300 mg/kg aqueous seed extract demonstrates a normal distribution of collagen fibres (red thin arrow) in the interstitial layer, similar to the control group.

Plates 4.3 (at 200x magnification) and 4.4 (at 400x magnification) illustrate the histoarchitecture of rat testis exposed to cadmium chloride toxicity and the protective effect of *Annona muricata*, as observed through Hematoxylin and Eosin staining. The control group displays a normal histomorphological appearance, with distinct spermatogonia cells (marked in red circles), sertoli cells (indicated by red arrowheads), seminiferous tubules (depicted with black thick arrows), and leydigs cells at the interstitial layer (highlighted by yellow thin arrows). In contrast, the group induced with cadmium chloride (CdCl2) exhibits severe degeneration and distortion of the testicular tissue, interstitial edoema (shown by black thin arrows), the formation of mitotic giant cells (indicated by red circles), congested tubules (depicted with black thick arrows), and an irregular distribution of spermatogonia (highlighted by yellow thin arrows) and Sertoli cells (indicated by red arrowheads). However, when treated with 100mg/kg aqueous seed extract, the group induced with cadmium chloride and treated shows congestion of the interstitial layer (marked by black thin arrows) with edema, irregular distribution of spermatogonia cells (shown by red circles), dilated seminiferous tubules (depicted with blue thick arrows), and reduced sperm cells. Similarly, the group induced with cadmium chloride and treated with 200mg/kg aqueous seed extract displays mild tissue regeneration, interstitial edoema (highlighted by yellow thin arrows) with reduced leydig cells, and dilated seminiferous tubules (indicated by black thick arrows). Lastly, the group induced with cadmium chloride and treated with 300mg/kg aqueous seed extract demonstrates a well-regenerated histological appearance, with well-differentiated and repaired spermatogonia cells (marked by red circles), sertoli cells (indicated by red arrowheads), and seminiferous tubules (depicted with yellow thick arrows).



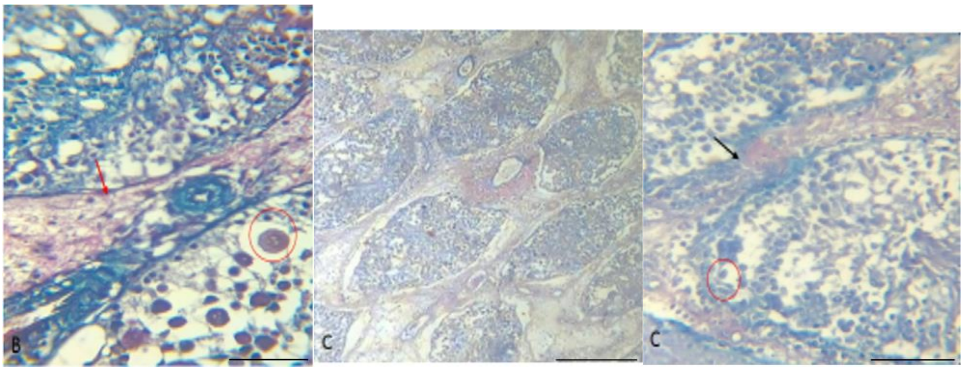


Plate 1, The control group shows a normal and well-differentiatedly distributed amount of collagen fibres in the interstitial layer (red thin arrow) between the seminiferous tubules, Induced with cadmium chloride (CdCl2), the group shows thickening of the tubular basement membrane of completely degenerated and atrophied seminiferous tubules with an excessive amount of collagenous fibres in the interstitial layer (red thin arrow) and the presence of multiple spermatid giant cells (red circle), Induced with cadmium chloride and treated with 100 mg/kg aqueous seed extract, *Annona muricata* shows mildly regenerated seminiferous tubules with thickened collagen fibre distribution at the interstitial layer (black thin arrow). MT 400x R, MT 200x L) Scale Bar =120 m,

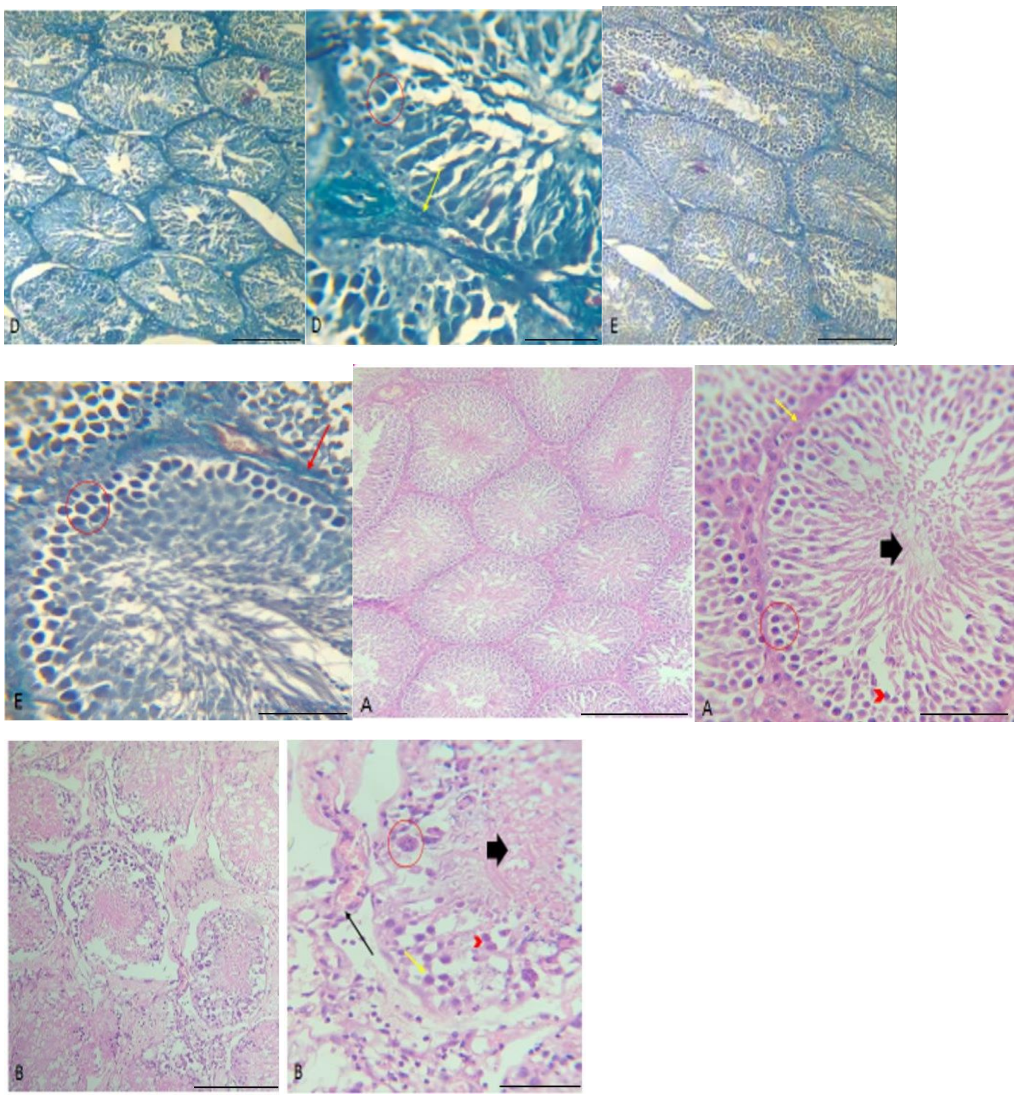


Plate 3 Induced and treated with 200mg/kg aqueous seed extract of Anona muricata shows a decrease in the deposition of collagen fibres (yellow thin arrow) in relation to the normal control, induced and treated with 300 mg/kg aqueous seed extract of Annona muricata, the normal distribution of collagen fibres (red thin arrow) in the interstitial layer is approximately similar to the control. In the control group showing normal histomorphological appearance, the spermatogonia cells (red circle), sertoli cells (red arrow head), seminiferous tubules (black thick arrow), and the leydigs cells at the interstitial layer (yellow thin arrow) are well

differentiated,induced with cadmium chloride (CdCl2) shows severe degeneration and distortion of the testicular tissue, interstitial edoema (black thin arrow), mitotic giant cell formation (red circle), congested tubules (black thick arrow), and irregular poikilocytic distribution of spermatogonia (yellow thin arrow) and sertoli cells (red arrow head). (HE 400x R, HE 200x L) Scale Bar =120 m

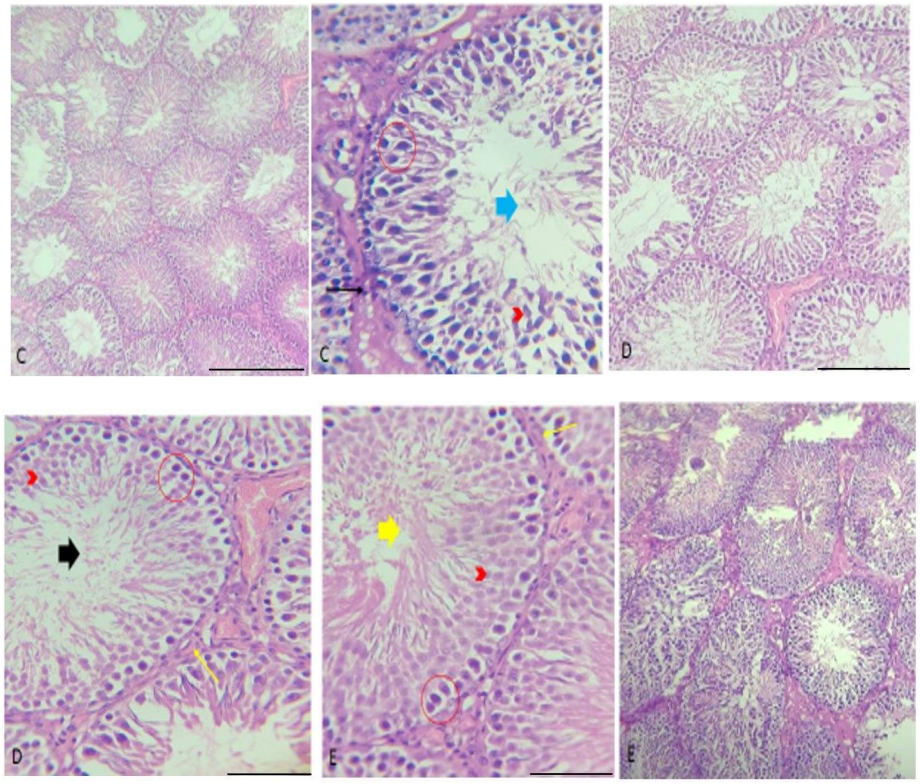


PLATE 4: Induced with cadmium chloride (CdCl2) and treated with 100mg/kg aqueous seed extract Annona muricata shows congestion of the interstitial layer (black thin arrow) with edoema, irregular distribution of spermatogonia cells (red circle), and dilated seminiferous tubules (blue thick arrow) with reduced sperm cells (HE 400x R, HE 200x L). Scale Bar =120 m, PLATE 9: Induced with cadmium chloride (CdCl2) and treated with 200mg/kg aqueous seed extract of Anons muricata shows mild tissue regeneration, interstitial edoema (yellow thin arrow) with reduced leydig cells, and dilated seminiferous tubules (black thick arrow) (HE 400x R, HE 200x L). Scale Bar =120 m, PLATE 10: Induced with cadmium chloride (CdCl2) and treated with 300mg/kg aqueous seed extract, the spermatogonia cells (red circle), sertoli cells (red arrow head), and seminiferous tubules (yellow thick arrow) are well differentiated and repaired. (HE 400x R, HE 200x L) Scale Bar =120 m

Discussion

Cadmium is a toxic heavy metal that can induce oxidative stress in various tissues, including the testes. Cadmium-induced oxidative stress occurs through multiple mechanisms that result in the accumulation of reactive oxygen species (ROS) and oxidative damage to cellular components. One of the primary mechanisms is the generation of ROS such as superoxide anion (O2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) (Fang et al., 2013; Ozcan and Ogun, 2015). Cadmium can increase the production of ROS by inducing the expression of enzymes such as NADPH oxidase, xanthine oxidase, and nitric oxide synthase. These enzymes are responsible for the production of ROS in different cellular compartments, such as the cytoplasm, mitochondria, and endoplasmic reticulum (Angeli et al., 2013). Cadmium also inhibits the activity of enzymes involved in the antioxidant defence system, such as superoxide dismutase (SOD) and catalase (CAT). SOD converts O2 into H_2O_2, which is further detoxified by CAT. Cadmium can directly inhibit the activity of these enzymes or indirectly by depleting the cellular levels of antioxidant molecules and leading to oxidative stress (Nikoli et al., 2016). This cadmium-induced oxidative stress was seen in our study according to table 2 above, where exposure to cadmium led to a decrease in SOD and CAT activity in the testis tissue. The results of this study correspond with those of Zhai et al. (2013), Merra et al. (2014), and Oladele et al. (2017). One of the mechanisms by which medicinal plants reduce oxidative stress is by increasing the levels of endogenous antioxidants. For example, plant-derived compounds such as flavonoids, carotenoids, and phenolic acids have been shown to upregulate the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (1). These enzymes convert harmful ROS into less harmful molecules, thus reducing oxidative stress (Palipoch, 2013). Medicinal plants also act by scavenging free radicals directly. Polyphenolic compounds found in different medicinal plants have been shown to neutralise free radicals by donating electrons or hydrogen atoms (Shebis et al., 2013). Also, previous studies have shown that the phytochemical screening of the seed extract of *A. muricata* revealed the presence of alkaloids, flavonoids, terpenoids, coumarins and lactones, anthraquinones, tannins, cardiac glycosides, phenols, phytosterols, and saponins (Gavamukulya*et al*., 2014). These phytochemicals may be responsible for the antioxidant and ant- inflammatory changes seen in Table 4.1. These results correspond with the results of Florence et al. (2014), Orak et al. (2019), and Roduan et al. (2019).

The tubular basement membrane (TBM) is a layer of extracellular matrix that surrounds the seminiferous tubules in the testis. It serves as a scaffold for the tubules and plays a crucial role in supporting the spermatogenesis process. In normal conditions, the TBM is thin and well organised (Al-Uboody, 2015). However, exposure to certain toxic substances such as cadmium can result in the thickening of the TBM, as seen in plates 4.1b and 4.2b above. Cadmium is a heavy metal that is widely distributed in the environment due to various industrial activities. It has been reported to cause testicular damage in animals and humans (Saleemi & Tahir, 2019). One of the pathological effects of cadmium exposure in the testis is the thickening of the TBM. This is thought to be due to the accumulation of collagen fibres and other extracellular matrix components in the TBM; this is also clearly seen in plates 4.1b and 4.2b above. The thickening of the TBM can impair the function of the seminiferous tubules by interfering with the exchange of nutrients and waste products between the blood vessels and the developing sperm cells. In severe cases, it can lead to the atrophy and degeneration of the seminiferous tubules (Anuales, 2016), which are responsible for the production of sperm. This pathological effect can be seen in Table 4.2. Moreover, the excessive amount of collagen fibers present in the interstitial layer of the testis due to cadmium exposure can further contribute to the degeneration and atrophy of the seminiferous tubules. Collagen fibres are a major component of the extracellular matrix and play an important role in tissue repair and remodelling (Halper

and Kjaer, 2014). However, the excessive deposition of collagen fibres in the interstitial layer can lead to fibrosis and impaired tissue function. In addition to the above, the presence of multiple spermatid giant cells in the histology of the testis is another pathological effect of cadmium exposure. Spermatid giant cells are abnormally large cells that are formed by the fusion of several immature sperm cells. They are typically observed in conditions where the spermatogenesis process is disrupted, such as in the case of cadmium toxicity.

The testicular interstitial tissue comprises blood vessels, lymphatic vessels, connective tissue, and endocrine cells, including Leydig cells. The interstitial tissue is critical for the normal functioning of the testis and plays a crucial role in the production of testosterone, the male sex hormone (Hedger, 2015). Cadmium-induced testicular interstitial edoema occurs as a result of damage to the blood vessels and capillaries in the interstitial tissue, leading to the leakage of fluid into the interstitial spaces. The edoema can lead to an increase in pressure in the interstitial tissue, which can further damage the blood vessels, reduce blood flow to the testis, and even affect the production of testosterone, leading to a drastic drop in the activity of testosterone as seen in figure 4.2, which will also lead to pathological changes in the weight of the testis (figure 4.1). The reduced blood flow and oxygen supply to the testis can result in impaired testicular function and reduced sperm production, leading to infertility. In addition, the increased pressure in the interstitial tissue can compress the seminiferous tubules, leading to their deformation and reduced sperm production, as seen in Table 4.2. *Annona muricata* is rich in antioxidants such as flavonoids, phenolic acids, and ascorbic acid, which are known to scavenge free radicals and prevent oxidative damage. Cadmium-induced testicular toxicity is mediated by the production of reactive oxygen species (ROS), which leads to lipid peroxidation and DNA damage (Hasmila et al., 2019). *Annona muricata* extracts have been shown to increase the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the testis, as seen in Table 4.1, thereby reducing oxidative stress and protecting against cadmium-induced damage, which will also have a protective effect on the histoarchitecture of the testis. Previous studies have also shown that *Annona muricata* extracts contain alkaloids such as annonacin, which has been reported to exhibit anti-inflammatory properties (Mutakin et al., 2022). Cadmium-induced testicular toxicity is associated with an increase in pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-), which promote inflammation and tissue damage. *Annona muricata* extracts have been shown to reduce the expression of these pro-inflammatory cytokines in the testis, thereby reducing inflammation and protecting against tissue damage, as seen in the results of our study. *Annona muricata* extracts contain bioactive compounds such as acetogenins, which have been reported to exhibit anti-cancer properties (Daud et al., 2016). Cadmium-induced testicular toxicity is associated with an increase in cell proliferation and apoptosis, which can lead to cancer. *Annona muricata* extracts have been shown to inhibit cell proliferation and induce apoptosis in the testis, thereby reducing the risk of cancer.

Conclusion

In summary, *Annona muricata* aqueous seed extract exerts its protective effect against cadmium-induced malignant testicular tumours by reducing oxidative stress, inflammation, and cell proliferation and apoptosis. These properties make it a promising therapeutic agent for the treatment of testicular toxicity induced by heavy metals such as cadmium.

Recommendation

The use of *Annona muricata* aqueous seed extract can provide a natural and safe alternative to alleviate the adverse effects of cadmium on the male reproductive system; further studies are

needed to evaluate the protective effect of other extracts from different parts of the *Annona muricata* plant against heavy metals.

References

Adelakun, S. A., Ogunlade, B., Olawuyi, T. S., Aniah, J. A., & Omotoso, O. D. (2018). Histomorphology, Sperm Quality and Hormonal Profile in Adult Male Sprague-Dawley Rats following administration of aqueous crude Extract of Solanum nigrum by gastric gavage. *JBRA Assisted Reproduction*, *22*(4), 338-345. https://doi.org/10.5935/1518-0557.20180051

Al-Uboody, W. S. H. (2015). *The Role of Quercetin against o-Anisidine Toxicity in Some Physiological, Biochemical and Histopathological Parameters of Laboratory Rats (Rattus norvegicus)* (Doctoral dissertation, Council of the College of Veterinary Medicine, University of Basrah).

Angeli, J. K., Pereira, C. A. C., de Oliveira Faria, T., Stefanon, I., Padilha, A. S., & Vassallo, D. V. (2013). Cadmium exposure induces vascular injury due to endothelial oxidative stress: the role of local angiotensin II and COX-2. *Free radical biology and medicine*, *65*, 838-848.

Anuales, X. J. (2016). XVIII ANNUAL MEETING OF THE ARGENTINE BIOLOGY SOCIETY. *Biocell*, *40*, 2.

Daud, N. N. N. N. M., Ya'akob, H., &Rosdi, M. N. M. (2016). Acetogenins of *Annona muricata* leaves: Characterization and potential anticancer study. *Integrative Cancer Science and Therapeutics*, *3*(4), 543-551.

Dimitriadis, F., Adonakis, G., Kaponis, A., Mamoulakis, C., Takenaka, A., &Sofikitis, N. (2017). Pre-testicular, testicular, and post-testicular causes of male infertility. Endocrinology of the testis and male reproduction. Cham: Springer, 1-47.

Fang, G. D., Zhou, D. M., & Dionysiou, D. D. (2013). Superoxide mediated production of hydroxyl radicals by magnetite nanoparticles: demonstration in the degradation of 2-chlorobiphenyl. *Journal of Hazardous Materials*, *250*, 68-75.

Florence, N. T., Benoit, M. Z., Jonas, K., Alexandra, T., Désiré, D. D. P., Pierre, K., & Théophile, D. (2014). Antidiabetic and antioxidant effects of *Annona muricata* (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. Journal of ethnopharmacology, 151(2), 784-790.

Gavamukulya, Y., Abou-Elella, F., Wamunyokoli, F., & AEl-Shemy, H. (2014). Phytochemical screening, anti-oxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). Asian Pacific journal of tropical medicine, 7, S355-S363.

Gezici, S., &Şekeroğlu, N. (2019). Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 19(1), 101-111.

Greenwell, M., & Rahman, P. K. S. M. (2015). Medicinal plants: their use in anticancer treatment. International journal of pharmaceutical sciences and research, 6(10), 4103.

Halper, J., & Kjaer, M. (2014). Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins. *Progress in heritable soft connective tissue diseases*, 31-47

Hasmila, I., Natsir, H., &Soekamto, N. H. (2019, October). Phytochemical analysis and antioxidant activity of soursop leaf extract (*Annona muricata* Linn.). In *Journal of Physics: Conference Series* (Vol. 1341, No. 3, p. 032027). IOP Publishing.

Hedger, M. P. (2015). The immunophysiology of male reproduction. *Knobil and Neill's physiology of reproduction*, 805.

Jurewicz, J., Radwan, M., Sobala, W., Radwan, P., Bochenek, M., & Hanke, W. (2014). Effects of occupational exposure-is there a link between exposure based on an occupational questionnaire and semen quality?. Systems biology in reproductive medicine, 60(4), 227-233.

Ibeh N. I., OmorodionN.T.(2017) Hibiscus flower extract (zobo)' as a moprhological counter stain for sperm cell analysis; adult male wister rats and rabbit as a model of study Enliven archive Microbiology Microbial Techniques 4(1): 001.

Lee, O. D., & Tillman, K. (2016). An Overview of Testosterone Therapy. American journal of men's health, 10(1), 68–72. https://doi.org/10.1177/1557988314556671

Merra, E., Calzaretti, G., Bobba, A., Storelli, M. M., & Casalino, E. (2014). Antioxidant role of hydroxytyrosol on oxidative stress in cadmium-intoxicated rats: different effect in spleen and testes. *Drug and Chemical Toxicology*, *37*(4), 420-426.

Meshram, P., & Pandey, B. D. (2019). Perspective of availability and sustainable recycling prospects of metals in rechargeable batteries–a resource overview. Resources Policy, 60, 9-22

Misra, H. P., &Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*, *247*(10), 3170-3175.

Mutakin, M., Fauziati, R., Fadhilah, F. N., Zuhrotun, A., Amalia, R., &Hadisaputri, Y. E. (2022). Pharmacological activities of soursop (*Annona muricata* Lin.). *Molecules*, *27*(4), 1201.

Nikolić, T. V., Kojić, D., Orčić, S., Batinić, D., Vukašinović, E., Blagojević, D. P., &Purać, J. (2016). The impact of sublethal concentrations of Cu, Pb and Cd on honey bee redox status, superoxide dismutase and catalase in laboratory conditions. *Chemosphere*, *164*, 98-105.

Omorodion, N.T., Aniobi C Chukwubuikem Uwadiegwu P Achukwu and Barisuka K NwibanaHepatocellular and renal effect of *GongronemaLatifolium*leaf extract on Albino rat following acetylaminophen (Paracetamol) poisoning, Nigerian Journal of Pharmaceutical and Applied Science Research, 10 (2): 21-27

Orak, H. H., Bahrisefit, İ. Ş., &Şabudak, T. (2019). Antioxidant activity of extracts of soursop (*Annona muricata* L.) leaves, fruit pulps, peels, and seeds. *Polish Journal of Food and Nutrition Sciences*.

Ozcan, A., & Ogun, M. (2015). Biochemistry of reactive oxygen and nitrogen species. *Basic principles and clinical significance of oxidative stress*, *3*, 37-58.

Palipoch, S. (2013). A review of oxidative stress in acute kidney injury: protective role of medicinal plants-derived antioxidants. *African Journal of Traditional, Complementary and Alternative Medicines*, *10*(4), 88-93

Rahman, M. (2017). Bioaccumulation and toxicity of cadmium, chromium and lead to live fish food, the tubificid worms (Tubifex spp.) (Doctoral dissertation, University of Dhaka).

Roduan, M. R. M., Abd Hamid, R., Cheah, Y. K., &Mohtarrudin, N. (2019). Cytotoxicity, antitumor-promoting and antioxidant activities of *Annona muricata* in vitro. *Journal of herbal medicine*, *15*, 100219.

Saalu L, Akunna G, and Ajayi J, (2013). Modulating Role of Bitter Leaf on Spermatogenic and Steroidogenesis Functions of the Rat Testis. American Journal of Biochemistry and Molecular Biology,3:314-321.doi: 10.3923/ajbmb.2013.314.321

Saleemi, M. K., Tahir, M. W., Abbas, R. Z., Akhtar, M., Ali, A., Javed, M. T., ... & Hassan, Z. U. (2019). Amelioration of toxicopathological effects of cadmium with silymarin and milk thistle in male Japanese quail (Coturnix japonica). *Environmental science and pollution research*, *26*, 21371-21380.

Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M. B., ... & Tauqeer, H. M. (2013). Heavy metals contamination and what are the impacts on living organisms. Greener Journal of Environmental management and public safety, 2(4), 172-179.

Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M. B., ... & Tauqeer, H. M. (2013). Heavy metals contamination and what are the impacts on living organisms. Greener Journal of Environmental management and public safety, 2(4), 172-179.

Sarkar, A., Ravindran, G., & Krishnamurthy, V. (2013). A brief review on the effect of cadmium toxicity: from cellular to organ level. Int J Biotechnol Res, 3(1), 17-36.

Sarkar, A., Ravindran, G., & Krishnamurthy, V. (2013). A brief review on the effect of cadmium toxicity: from cellular to organ level. Int J Biotechnol Res, 3(1), 17-36.

Seed, J., Chapin, R. E., Clegg, E. D., Dostal, L. A., Foote, R. H., Hurtt, M. E., ... & Wise, L. D. (1996). Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. *Reproductive toxicology*, *10*(3), 237-244.

Sengupta, P., Borges Jr, E., Dutta, S., & Krajewska-Kulak, E. (2018). Decline in sperm count in European men during the past 50 years. Human & experimental toxicology, 37(3), 247-255.

Shebis, Y., Iluz, D., Kinel-Tahan, Y., Dubinsky, Z., & Yehoshua, Y. (2013). Natural antioxidants: function and sources.

Sherif, H. B., Baba, G., & Abdullahi, S. M. (2017). Acute and sub-chronic toxicity profile of Annona muricata (Sour sop) on wister albino rats. *Bayero Journal of Pure and Applied Sciences*, *10*(2), 57-63.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical biochemistry*, *47*(2), 389-394.

Tiwana, M. S., & Leslie, S. W. (2021). Anatomy, Abdomen and Pelvis, Testicle. In StatPearls. StatPearls Publishing

Zhai, Q., Liu, Y., Wang, C., Zhao, J., Zhang, H., Tian, F., ... & Chen, W. (2019). Increased cadmium excretion due to oral administration of Lactobacillus plantarum strains by regulating enterohepatic circulation in mice. Journal of agricultural and food chemistry, 67(14), 3956-3965.

Zhai, Q., Wang, G., Zhao, J., Liu, X., Tian, F., Zhang, H., & Chen, W. (2013). Protective effects of Lactobacillus plantarum CCFM8610 against acute cadmium toxicity in mice. *Applied and environmental microbiology*, *79*(5), 1508-1515.

Zhang, H., Yan, J., Xie, Y., Chang, X., Li, J., Ren, C., ... & Li, X. (2022). Dual role of cadmium in rat liver: Inducing liver injury and inhibiting the progression of early liver cancer. Toxicology Letters, 355, 62-81.

Zhang, Z., Zheng, Z., Cai, J., Liu, Q., Yang, J., Gong, Y., ... & Xu, S. (2017). Effect of cadmium on oxidative stress and immune function of common carp (Cyprinus carpio L.) by transcriptome analysis. Aquatic Toxicology, 192, 171-177.