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EFFECTS OF EDIBLE BIRD'S NEST ON OVARY AND UTERUS OF CYCLING SPRAGUE DAWLEY RATS SUBJECTED TO CADMIUM TOXICITY

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

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Chairman : Associate Professor Nurhusien Yimer Degu, PhD
Faculty : Veterinary Medicine

Cadmium (Cd), an abundant heavy metal which is continually released into the environment by human economic activities, causes severe health damages. Humans and animals are mainly exposed to this toxic metal through occupation, diet, respiration, smoking, and water. Various studies on female rats have revealed that Cd accumulates in the female reproductive tract with a considerably quite long biological half-life and causes reproductive dysfunctions. The edible bird's nest (EBN) is made from the salivary secretions of male swiftlet birds (*Aerodramus fuciphagus* and *Aerodramus maximus*). EBN is traditionally consumed for its medicinal and nutritional values. As of today, no prior studies have detailed out the effects of EBN on Cd-mediated reproductive toxicity in female animals. Therefore, this study was designed to investigate EBN's ameliorating role against Cd toxicity induced reproductive dysfunction in cycling female rats.

Thirty (30) female Sprague Dawley rats were assigned into five groups as follows: group 1, control (C) received distill water; treatment group 2 (T0) was administered with $CdCl_2$ (5mg/kg BW); while group 3 (T1), group 4 (T2) and group 5 (T3) were administered with $CdCl_2$ (5mg/kg BW) and graded concentrations of 60, 90 and 120 mg/kg BW of EBN via oral gavage respectively. After four weeks of the challenge, the experimental rats were euthanised under general anesthesia for blood and Uterine and Ovarian tissue sample collection. Histomorphometric analysis of the tissues were employed using H&E staining, while assessment of expressions of metallothionein (MT), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) were assessed by using Immunohistochemistry and measurement of plasma levels of estradiol (E_2) and progesterone (P_4) were done by using ELISA, Cd levels in uterine and ovarian tissues were assessed through ICP-MS.

Oral administration of cadmium chloride ($CdCl_2$) without EBN supplement (T0) resulted in significantly ($p<0.05$) higher accumulation of Cd (238.9 ± 23.7, 237.9 ± 37.3 ppb) in uterine and ovarian tissues respectively compared with the group C (3.3 ± 0.5, 4.2 ± 0.4) and other treatment groups (T1: 125.4 ± 16.1, 99.2 ± 5.9; T2: 89.3 ± 15.6, 84.8 ± 6.0; T3: 65.7 ± 12.0, 41.3 ± 3.6 ppb). The deposition of Cd in both tissues appeared to decrease with EBN supplement in a dose dependent manner. Meanwhile, increased immunohistochemical expressions of MT in uterine and ovarian tissues as assessed by number of positive stained cells was found in T0 compared to the C and other treatment groups (Uterus= C: 55 ± 2; T0: 109.3 ± 2.1; T1: 87 ± 2.5; T2: 78.3 ± 2.0; T3: 62.3 ± 0.8; Ovaries= C: 0.3 ± 0.3; T0: 3.0 ± 3.0; T1: 1.3 ± 0.3, T2: 0.6 ± 0.3, T3: 0.3 ± 0.3, staining intensity, $p<0.05$). On the other hand, a significant decrease ($p<0.05$) in the activity of superoxide dismutase (SOD, µ/mL) in T0 (0.0783± 0.0017) compared with the C (0.180 ± 0.001) and EBN supplemented groups (T1: 0.1 ± 0.0013; T2: 0.1 ± 0.0016; T3: 0.1 ± 0.0021) were found. There was a significantly ($p<0.05$) evident increase in thiobarbituric acid reactive substance (TBARS) levels in Cd only treated group as compared with negative control and EBN supplemented groups (C: 30.98 ± 2.7; T0: 35.8 ± 3.09; T1: 33.8± 2.18; T2: 25.85 ± 3.7; T3: 23.4 ± 3.7, nmol/mL). Moreover, the Cd only treated group revealed uterine histopathological changes which include cystic glands, loss of normal structure of luminal epithelium (LE) and glandular epithelium (LE) cells. While animals treated with Cd and EBN resulted in a significantly low level ($p<0.05$) of Cd in uterus and ovaries and lower uterine MT expression, lower degenerative changes of the LE and GE cells with normal histomorphology of glands as well as increased antioxidant SOD activity compared to Cd only treated group. Animals administered with only Cd resulted in decreased immunohistochemical expressions of VEGF (C: 86.33± 1.5; T0: 84.66± 3.17; T1: 108.3± 4.3; T2: 122.66± 4.9; T3: 132 ± 4.58, no of deterred cel +ve stained cells, $p<0.05$) , EGF (C: 80.66± 3.5; T0: 73 ± 2.64; T1: 93.33 ± 2.7; T2: 115.33 ± 2.33; T3: 121 ± 3, no of +ve stained cells, $p<0.05$) and EGFR (C: 96.33± 3.2; T0: 67.8 ± 0.98; T1: 108.33± 4.37; T2: 122.66 ± 4.91; T3: 141.3 ± 3.28, no of positive stained cells, $p<0.05$) in uterine tissues.. While animals treated with $CdCl_2$ and EBN at three different dosages resulted in higher VEGF, EGF and EGFR expressions compared to Cd only treated group. The higher degree expressions for the growth factors (VEGF, EGF, EGFR) in the EBN supplemented groups compared to even the untreated control group reflects the strong ameliorating effect of EBN that surpasses the toxic effect of Cd exposure. The respective plasma concentrations of E_2 and P_4 (ng/L) in all treated groups (T0: 3.8± 0.1, 2.8± 0.1; T1: 4.5± 0.3, 4.5± 0.2; T2: 5.6± 0.1, 5.6± 0.2; T3: 7.0 ± 0.3, 6.1± 0.3) decreased significantly ($p<0.05$) in comparison with the control (C: 9.8± 0.3, 6.7± 0.3). Concentrations of E_2 was significantly higher ($p<0.05$) in T3 as compared to other treated groups. Plasma P_4 concentration decreased significantly in T0 as compared to control. The plasma P_4 concentration in all EBN treated groups was significantly increased compared with the Cd-only group; with T3 showing a significantly higher ($p<0.05$) concentration. Meanwhile, $CdCl_2$ found to have no significant impact on the estrous cycle length (4-5 days) across experimental groups; cells from vaginal smear showed normal morphology.

Overall, the findings of this study revealed that oral exposure to Cd at a dose of 5 mg/kg BW results in significant alterations in the uterus and ovaries as evidenced by high Cd levels in these tissues and higher degree ($p<0.05$) of MT expression along with reduced antioxidant activity and histomorphological changes. Meanwhile, EBN proved to play a significant protective role against Cd-induced reproductive toxicity; the protection was

higher at the dose rate of 120mg/kg. The low level of Cd deposition paralleled with reduced degree of MT expressions found in both the uterine and ovarian tissues of EBN supplemented groups that lead to an interesting conclusion of EBN's potential role as a chelating agent for Cd, though its mechanism is something to be explored in future. In general, this study suggests that EBN has an ameliorating effect against Cd-induced reproductive toxicity. It protects and improves functions of the uterus and ovaries through its potent antioxidant activity, enhancing expressions of growth factors (EGF, EGFR, and VEGF), prevention of Cd deposition along with a rise in plasma levels of E_2 and P_4.

Keywords: Edible bird nest, Cadmium toxicity, Uterus, Ovaries, Growth factors, Oxidative stress, steroid hormones.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

KESAN SARANG BURUNG BOLEH MAKAN TERHADAP KAJIAN KETOKSIKAN KADMIUM KE ATAS ESTRUS PADA TIKUS SPRAGUE DAWLEY

Oleh

QUDDUS ABDUL

Januari 2021

Pengerusi : Profesor Madya Nurhussein Yimer Degu, PhD
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Kadmium (Cd), merupakan logam berat yang amat banyak dilepaskan secara berterusan ke alam sekitar melalui aktviti manusia mengakibatkan kemudaratan yang teruk. Manusia dan haiwan sellau terdedah kepada logam toksik ini melalui pekerjaan, pemakanan, pernafasan, merokok dan air. Banyak kajian pada tikus betina menunjukkan bahawa pengumpulan Cd dengan jangka hayat panjang pada trakus pembiakan tikus betina dan membawa kepada disfungsi pembiakan. Sarang burung boleh makan (EBN) dihasilkan dari rembesan air liur burng walit jantan (Aerodramus fuciphagus dan Aerodramus maximus). Secara tradisi, EBN diambil kerana nilai perubatan dan pemakanannya. Sehingga kini, tiada ujian sebelum ini memperincikan kesan EBN terhadap ketoksikan berantara-Cd pada haiwan betina. Dengan itu kajian ini direka untuk menyiasat peranan pemulihan EBN terhadap aruhan ketoksikan Cd pada disfungsi pembiakan tikus betina utuh.

Sebanyak tiga puluh ekor (30) tikus betina Sprague Dawley diasingkan kepada lima kumpulan seperti berikut: C, T0 yang masing-masing menerima secara gavaj oral air suling, CdCl2 (5mg/kg BW). Selain dari menerima CdCl2 (5mg/kg BW), penambahan EBN sebanyak 60, 90 and 120 mg/kg BW masing-masing kepada kumpulan T1, T2 dan T3. Empat minggu pasca-cabaran, kesemua tikus dieutanasia sebelum darah, uterus dan ovari diambil. Analisis histomorfometri sampel tisu menggunakan pewarna H&E, penilaian penyertaan imunohistokimia metalothionein (MT), faktor pertumbuhan endothelium vesel (VEGF), pertumbuhan epidermis (EGF) and reseptor pertumbuhan epidermis (EGFR), pengukuran aras estradiol (E2) plasma dan progesteron (P4) menggunakan ELISA selain kepekatan Cd dalam uterus and ovari secara ICP-MS.

Pemberian CdCl2 tanpa penambahan EBN (T0) mengakibatkan pengumpulan tertinggi Cd, pernyataan MT, aras TBARS serta lesi ($p<0.05$) pada uterus dan ovari berbanding kumpulan lain. Pemendapan Cd dan pernyataan MT dalam kedua tisu berpola menurun menurut dos dengan penambahan EBN. Bagaimanapun, aras aktiviti superoksida dismutase (SOD, μ/mL)serta pernyataan VEGF, EGF, EGFR adalah terendah pada kumpulan T0. Pernyataan tinggi VEGF, EGF, EGFR pada kumpulan penambahan EBN menandakan kesan positif EBN mengurangkan kesan toksik pendedahan kepada Cd. Kepekatan E2 and P4 pada kesemua kumpulan rawatan adalah terendah ($p<0.05$) berbanding kumpulan C. Bagaimanapun, kepekatan E2 pada kumpulan T3 adalah lebih tinggi ($p<0.05$) daripada kumpulan T0, T1 dan T2. Kepekatan plasma P4 pada kumpulan T0 lebih rendah ($p<0.05$) dari kumpulan C kecuali kesemua kumpulan penerima EBN yang lebh tinggi dari kumpulan T0. Adalah didapati bahawa Cd tiada mempunyai kesan ketara terhadap sela kitaran estrus (4-5 days) pada kesemua kumpulan dengan calitan morfologi vagina yang normal.

Secara am, pendedahan kepada Cd pada dos 5 mg/kg BW menyebabkan kesan ketara pada uterus and ovari yang dapat diperbaiki dengan pemberian EBN. Dengan itu, EBN berupaya berperanan sebagai agen pengkelat Cd yang memerlukan kajian lanjut bagi menerangkan mekanismenya. Ia juga melindungi dan meningkatkan fungsi uterus dan ovari melalui aktiviti antipengoksid, memudahkan pernyataan EGF, EGFR dan VEGF serta melindungi tisu dari pemendapan Cd dan meningkatkan aras plasma E2 dan P4.

Kata Kunci**:** Sarang burung boleh makan, Kadmium, Uterus, Ovari, faktor pertumbuhan, tekanan oksidatif

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LIST OF ABBREVIATIONS

%	Percentage
µ/mg	Micro/milligram
µm	Micrometer
ALP	Alkaline phosphatase
APP	amyloid precursor protein
ALT	Alanine transaminase
BBB	Blood brain barrier
BUN	Blood Urea Nitrogen
BW	Body weight
CAT	Catalase
Cd	Cadmium
CdO	Cadmium oxide
CNS	Central nervous system
COX-2	cyclooxygenase 2
CRE	Creatinine
DNA	Deoxyribonucleic acid
DW	Distilled water
E_2	Estrogen
EBN	Edible bird's nest
EGF	Epidermal growth factor
EGFR	epidermal growth factor receptor
FSH	Follicular stimulating hormone
g	Gram

GAGs	Glycosaminoglycan chondroitin
GE	Glandular epithelium
GSH	glutathione
H & E	Hematoxylin and Eosin
HCL	Hydrochloric acid
HNO_3	Nitric acid
IACUC	International Animal Care Use Committee
IgE	Immunoglobulin E
Kg	Kilogram
LE	Luminal epithelium
LH	Luteinizing Hormone
MAPK	mitogen-activated protein kinase
MDA	Malondialdehyde
MT	Metallothionein
mg	Milligram
Min	Minute
mL	Milliliter
OD	Optical density
OECD	Organization economic committee development
P	Prolactin
P_4	Progesterone
Pb	Lead
PBS	phosphate buffer saline
pH	potential of Hydrogen

PLT	Platelet
PMN	Polymorphonuclear neutrophils
RBC	red blood cell
ROS	Reactive oxygen species
SOD	superoxide dismutase
T	Testosterone
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substance
TNF-α	tumor necrosis factor-alpha
UPM	Universiti Putra Malaysia
VEGF	Vascular endothelial growth factor
WHO	World health organization

CHAPTER 1

INTRODUCTION

1.1 Overview

In recent decades, a great deal of attention has been given to the biochemical functions of natural substances in biological systems (Brzóska *et al*., 2016). Various plant and animal origin compounds are reported to play a protective role against heavy metal toxicity (Dailiah & Padmalatha, 2012). Edible bird's nest (EBN), the nest made from the salivary secretions of swiftlet bird, has been consumed as a tonic or healthy food for decades. The major source of swiftlets is found in Indonesia, while Malaysia's Sarawak and Sabah provinces are the second-largest swiftlet contributors (L. S. Chua & Zukefli, 2016). The species found in Malaysia are *Hydrochus gigas*, *Collocalia esculent*, *Cypsiurus balasiensis* (American Swift Palm), *Aerodramus. maximus*, and *Aerodramus. fuciphagus* (Jamaluddin *et al*., 2019a). *A. maximus* and *A. fuciphagus* are harvested on a large scale for trade and commercial purposes, particularly during the breeding season that is reported from the month of November to March (Ma & Liu, 2012).

EBN has been reported to have a wide range of medicinal benefits including, enhancing the immune system, complexion, and stimulating epidermal growth and improving respiratory problems, inhibiting viral infections, and inhibition of apoptosis (Yew *et al*., 2014; Dai *et al*., 2020). The fermentation of EBN glycan and glycopeptide has contributed several profiles of the gut bacterial growth that may imply various effects in the gut environment (Aliah Daud *et al*., 2019). An EGF-like component found in EBN has also been associated with its role in cell division, growth, and tissue regeneration enhancement (Dai et al., 2020). This phenomenon has been regarded as one of the factors for the rejuvenating properties of EBN (L. S. Chua & Zukefli, 2016). A recent study revealed that EBN significantly improved memory and neuroprotection by inhibiting neuroinflammatory and oxidative stress processes (Careena *et al*., 2018). EBN contains VEGF and IL-6 which prevents the apoptosis of embryonic neurons by inhibiting the activation of caspase three, leading to the suppression of apoptotic cells (Roh *et al*., 2012). A study conducted by Ma & Liu (2012) confirmed that EBN contains reproductive hormones such as testosterone, FSH, LH, E_2, and P_4. Whereas, up-regulation of VEGF expression by E_2 leading to increased angiogenesis has been previously reported (Hervé *et al*., 2006). Recent studies demonstrated that EBN increases the fertility rate and the rate of embryo implantation by enhancing the proliferation and differentiation of uterine structures, as shown by steroid receptor expressions up-regulation (Albishtue *et al*., 2019).

Cd is an abundant heavy metal that causes toxicity in various organs. It is continually released into the atmosphere due to human economic activities such as refining and smelting of non-ferrous metals, phosphate fertilizers, batteries manufacturing, recycling of electronic and metal waste, and incineration of municipal waste (Tchounwou *et al*.,

2012; Turner, 2019; Zhang & Reynolds, 2019). Cd usually enters the body, mainly due to work exposure, diet, respiration, smoking, and water (Vardhan *et al*.2019). The stated heavy metal has an extremely long biological half-life, which is between 15 to 30 years (Satarug *et al*.2010). According to WHO (2010), the levels of Cd have increased currently in the environment, workplace, and food chains due to anthropogenic practices and its widespread use in commercial products such as rechargeable batteries, pigments, vacuum tubes, some lubricants and nanosized particles of cadmium oxide (CdO) (Turner, 2019). This toxicity may cause damages to various tissues and organs, such as in the kidneys, liver, lungs, bones, and brain (Geng & Wang, 2019). Several studies have indicated that Cd's has endocrine modulative properties. Therefore it has been included in the category of endocrine disruptors, which are known as B exogenous mixtures or compounds that disrupt endocrine system functions and cause harmful health effects in an organism or its progeny or different populations (Epidemic, 2017). Many studies have outlined the impact of Cd on gametogenesis in both females and males, and implicating its compounds in early implantation failure and embryo lethality (Thompson & Bannigan, 2008). Various studies on female rats revealed that with a considerably quite long half-life, it accumulates in the female reproductive tract (Nasiadek *et al*., 2011). It has caused endometriosis in female rats (Nasiadek *et al*., 2018). Moreover, the number of uterine implantation sites and uterine length were decreased as a result of Cd exposure (Henson & Chedrese, 2004). Cd nanoparticles may alter reproductive success and perinatal growth and development as these nanoparticles can reach the placenta and lead to an unfavorable environment for the developing fetus (Blum *et al*.,2012).

Research findings on EBN have demonstrated that it enhances the reproductive functions and increases the rate of a successful pregnancy; furthermore, it has shown a protective effect against reproductive damages caused by heavy metal lead acetate (Albishtue *et al*. 2018; Albishtue *et al*. 2019). EBN also contains many other biological properties such as the potential to stimulate proliferation and growth of stem cells, EGF-like activity, enhance the biosynthesis of reproductive hormones like estrogen and act as an antioxidant. All these biological properties of EBN may influence the reproduction process. Consumers flock to EBN as compared to other natural products because of its high nutritional values such as anti-aging, immunomodulatory, and antioxidant effects. Despite the potential biological roles EBN has got (Chua et al., 2013, Akmal et al., 2017, Albishtue et al., 2018), research on effect of EBN supplement on reproduction/fertility is limited. EBN has been praised for its strong potential to be used as a hormonal replacement prophylaxis without any reported side effects(Zhiping et al., 2015), while several other types of hormonal replacement agents may lead to the development of endometrial and breast cancer (Zuccheto et al., 2009).

1.2 Problem statement

The forecasts indicate a rise in environmental contamination with Cd, and thus there will be an increase in exposure to the general population. Human exposure to Cd is currently a severe concern in fast-developing countries (Anetor, 2012). Food is the primary source of the general population's exposure to this toxic element, while habitual smoking of tobacco is also considered as a major source of exposure to Cd (Järup *et al*. 2015). Smoking cigarettes is an increasing pandemic in Malaysia (Lee, 2014). A cross-sectional

study by Lim *et al*. (2018) with representative sample of 21445 adults showed that the overall prevalence of smoking was 22.8%. The accumulation of Cd in urine was correlated with smoking habits among the Malaysian population (Adnan *et al*., 2012; Ismail *et al*., 2018).

The low-level lifetime exposure to this toxic metal may lead to damage to the liver, kidneys, cardiovascular system, and skeletal system, as well as to the deterioration of the hearing and sight (Järup, 2003; Brzóska & Moniuszko-Jakoniuk, 2004; Wallin *et al*., 2014). Moreover, it can alter reproduction and development in various ways at every stage of the reproductive process (Thompson & Bannigan, 2008). Several short and long-term studies have shown the detrimental effects of Cd-exposure on both female and male reproductive functions (Chedrese *et al*.2008). Long term exposure to this heavy metal may lead to reproductive function disorders, which might even lead to infertility (Nasiadek *et al*. 2019).

Considering all the bioactive constituents and functions of EBN, we hypothesized that EBN would confer ameliorating effects against reproductive changes caused by Cd in uterus and ovaries of cycling female Sprague Dawley rats. As of today, no prior studies on the impact of EBN on Cd-mediated reproductive toxicity in female rats exist. Based on these observations, this study was designed to investigate whether EBN (*Aerodramus fuciphagus*) has an ameliorating effect on Cd exposure in the uterus and ovary of an experimental rat model.

1.3 Research objectives

1 To investigate the protective role of EBN supplement on uterine and ovarian histomorphology in non-pregnant cycling rats subjected to cadmium toxicity.

2 To evaluate the ameliorating effect of EBN supplement on endometrial expressions of metallothionein (MT), growth factors and Cd-accumulation in uterus and ovaries of cycling rats exposed to Cd-toxicity.

3 To assess the attenuating effect of EBN supplement against oxidative stress and hormonal (E_2 and P_4) imbalance in blood plasma of cycling rats subjected to Cd toxicity and sacrificed at the stage of estrus.

1.4 Research hypothesis

Objective 1:

- H_0 = EBN supplement has no significant ameliorating effect against uterine and ovarian histomorphological change caused by cadmium toxicity.
- H_1= EBN supplement has significant ameliorating effect against uterine and ovarian histomorphological changes caused by cadmium toxicity

Objective 2:

- H_0 = EBN supplement has no significant effect on expressions of growth factors and metallothionein in uterine and ovarian tissues of Cd-intoxicated rats.
- H_1= EBN supplement has significant effect on expressions of growth factors and metallothionein in uterine and ovarian tissues of Cd-intoxicated rats
- H_0 = Cadmium accumulation in the uteri and ovaries of rats is not altered by EBN supplement.
- H_1= = Cadmium accumulation in the uteri and ovaries of rats is altered by EBN supplement.

Objective 3:

- H_0 = Plasma levels of oxidative stress biomarkers, antioxidants, and steroid hormones in rats exposed to cadmium toxicity are not affected by EBN supplement.
- H_1= Plasma levels of oxidative stress biomarkers, antioxidants, and steroid hormones in rats exposed to cadmium toxicity are affected by EBN supplement.

REFERENCES

Abbas, K., Monaghan, S. D., & Campbell, I. (2019). Uterine physiology. *Anaesthesia and Intensive Care Medicine*, *20*(7), 389–391. https://doi.org/10.1016/j.mpaic.2019.04.003

Abdeen, A., Ghonim, A., El-Shawarby, R., Abdel-Aleem, N., El-Shewy, E., Abdo, M., & Abdelhiee, E. (2017). Protective effect of cinnamon against cadmium-induced hepatorenal oxidative damage in rats. *International Journal of Pharmacology and Toxicology*, *5*(1), 17. https://doi.org/10.14419/ijpt.v5i1.7119

Abubakar, K., Mailafiya, M. M., Danmaigoro, A., Chiroma, S. M., Rahim, E. B. A., & Zakaria, M. Z. A. B. (2019). Curcumin attenuates lead-induced cerebellar toxicity in rats via chelating activity and inhibition of oxidative stress. *Biomolecules*, *9*(9). https://doi.org/10.3390/biom9090453

Adnan, J. A., Azhar, S. S., Hasni, J. M., & Ahmad, J. S. (2012). Urinary cadmium concentration and its risk factors among adults in Tanjung Karang, Selangor. *American-Eurasian J. Toxicol. Sci*, *4* (June 2014), 80–88. https://doi.org/10.5829/idosi.aejts.2012.4.2.6331

Aimola, P., Carmignani, M., Volpe, A. R., Di Benedetto, A., Claudio, L., Waalkes, M. P., van Bokhoven, A., Tokar, E. J., & Claudio, P. P. (2012). Cadmium induces p53-dependent apoptosis in human prostate epithelial cells. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0033647

Akmal, M., Intan-Shameha, A., Ajat, M., & Ideris, A. (2017). *Edible Bird'S Nest (Ebn) Is a Potential Natural Product Against Influenza Virus Infection*. *29*(2), 14–19.

Al-hashem, F., Dallak, M., Bashir, N., & Abbas, M. (2009). Camel ' s Milk Protects Against Cadmium Chloride Induced Toxicity in White Albino Rats Riyadh Elessa , 4 Mohammad Khalil and 5 Mahmoud Al-Khateeb Department of Physiology , College of Medicine , King Khalid University , Abha 64121 , Saudi Arabia Departmen. *American Journal of Pharmacology and Toxicology*, *4*(3), 107–117.

Albishtue, A. A., Almhanna, H. K., Yimer, N., Zakaria, M. Z. A., Haron, A. W., & Almhanawi, B. H. (2020). Effect of edible bird's nest supplement on hepato-renal histomorphology of rats exposed to lead acetate toxicity. *Jordan Journal of Biological Sciences*, *13*(2), 213–218.

Albishtue, A. A., Yimer, N., Zakaria, M. Z. A., Haron, A. W., Babji, A. S., Abubakar, A. A., & Almhanawi, B. H. (2018). Effects of EBN on embryo implantation, plasma concentrations of reproductive hormones, and uterine expressions of genes of PCNA, steroids, growth factors and their receptors in rats. *Theriogenology*. https://doi.org/10.1016/j.theriogenology.2018.12.026

Albishtue, A. A., Yimer, N., Zakaria, M. Z. A., Haron, A. W., Babji, A. S., Abubakar, A. A., & Almhanawi, B. H. (2019). Effects of EBN on embryo implantation, plasma concentrations of reproductive hormones, and uterine expressions of genes of PCNA, steroids, growth factors and their receptors in rats. *Theriogenology*, *126*, 310–319. https://doi.org/10.1016/j.theriogenology.2018.12.026

Albishtue, A. A., Yimer, N., Zakaria, M. Z. A., Haron, A. W., Babji, A. S., Abubakar, A. A., Baiee, F. H., Almhanna, H. K., & Almhanawi, B. H. (2019). The role of edible bird's nest and mechanism of averting lead acetate toxicity effect on rat uterus. *Veterinary World*, *12*(7), 1013–1021. https://doi.org/10.14202/vetworld.2019.1013-1021

Albishtue, A. A., Yimer, N., Zakaria, M. Z. A., Haron, A. W., Yusoff, R., & Almhanawi, B. H. (2018). Ameliorating effect of edible bird's nest against lead acetate toxicity on the rat hypothalamic–pituitary–ovarian axis and expressions of epidermal growth factor and vascular endothelial growth factor in ovaries. *Comparative Clinical Pathology*, *23*(3), 1–11. https://doi.org/10.1007/s00580-018-2729-y

Albishtue, A. A., Yimer, N., Zakaria, M. Z. A., Haron, A. W., Yusoff, R., Assi, M. A., & Almhanawi, B. H. (2018). Edible bird's nest impact on rats' uterine histomorphology, expressions of genes of growth factors and proliferating cell nuclear antigen, and oxidative stress level. *Veterinary World*, *11*(1), 71–79. https://doi.org/10.14202/vetworld.2018.71-79

Albishtue, A. A., Yimer, N., Zakaria, Z. A., Haron, A. W., & Yusoff, R. (2018). Ameliorating effect of edible bird ' s nest against lead acetate toxicity on the rat hypothalamic – pituitary – ovarian axis and expressions of epidermal growth factor and vascular endothelial growth factor in ovaries. *Comparative Clinical Pathology*, *1*, 1257–1267.

Alkhedaide, A., Alshehri, Z. S., Sabry, A., Abdel-Ghaffar, T., Soliman, M. M., & Attia, H. (2016). Protective effect of grape seed extract against cadmium-induced testicular dysfunction. *Molecular Medicine Reports*, *13*(4), 3101–3109. https://doi.org/10.3892/mmr.2016.4928

Alpsoy, S., Kanter, M., Aktas, C., Erboga, M., Akyuz, A., Akkoyun, D. C., & Oran, M. (2014). Protective effects of onion extract on cadmium-induced oxidative stress, histological damage, and apoptosis in rat heart. *Biological Trace Element Research*, *159*(1–3), 297–303. https://doi.org/10.1007/s12011-014-9968-9

Amamou, F., Nemmiche, S., Meziane, R. kaouthar, Didi, A., Yazit, S. M., & Chabane-Sari, D. (2015). Protective effect of olive oil and colocynth oil against cadmium-induced oxidative stress in the liver of Wistar rats. *Food and Chemical Toxicology*, *78*, 177–184. https://doi.org/10.1016/j.fct.2015.01.001

Amit, P., Ranjan, D. S., Sutapa, B., & Sukumar, K. (2019). Study on the effects of cadmium chloride on liver and testis in albino rats. *Research Journal of Chemistry and Environment*, *23*(8), 85–93.

Anetor, J. I. (2012). Rising environmental cadmium levels in developing countries: Threat to genome stability and health. *Nigerian Journal of Physiological Sciences*, *27*(2), 103–115. https://doi.org/10.4172/2161-0525.1000140

Angelis, C. De, Galdiero, M., Pivonello, C., Salzano, C., Gianfrilli, D., Piscitelli, P., Lenzi, A., Colao, A., & Pivonello, R. (2017). The environment and male reproduction : The effect of cadmium exposure on reproductive function and its implication in fertility. *Reproductive Toxicology*, *73*, 105–127. https://doi.org/10.1016/j.reprotox.2017.07.021

Arroyo, V. S., Flores, K. M., Ortiz, L. B., Gómez-quiroz, L. E., & Gutiérrez-ruiz, M. C. (2013). Liver and Cadmium Toxicity. *Journal of Drug Metabolism & Toxicology*, *03*(06), 1–7. https://doi.org/10.4172/2157-7609.s5-001

Atef, M. M. A., Fatma, A. A. I., Noha, A. A. E.-L., Samir, W. A., & Sherif, A. A. M. (2014). Protective effects of ginger (Zingiber officinale Roscoe) against cadmium chloride-induced oxidative stress in the blood of rats. *Journal of Medicinal Plants Research*, *8*(39), 1164–1172. https://doi.org/10.5897/jmpr2014.5531

Atsukawa, N. M., Atsumoto, M. M., Ukawa, W. B., Hiji, H. C., Akayama, K. N., Ara, H. H., & Sukahara, T. T. (2011). *Improvement of Bone Strength and Dermal Thickness Due to Dietary Edible Bird ' s Nest Extract in Ovariectomized Rats*. *75*(3), 590–592. https://doi.org/10.1271/bbb.100705

Babji, A. S., Nurfatin, M. H., Etty Syarmila, I. K., & Masitah, M. (2015). Secrets of Edible Bird Nest. *Agricultural Science Journal*, *1*(1), 32–37.

Bagchi, D., Joshi, S. S., Bagchi, M., Balmoori, J., Benner, E. J., Kuszynski, C. A., & Stohs, S. J. (2000). Cadmium- and chromium- induced oxidative stress, DNA damage, and apoptotic cell death in cultured human chronic myelogenous leukemic K562 cells, promyelocytic leukemic HL- 60 cells, and normal human peripheral blood mononuclear cells. *Journal of Biochemical and Molecular Toxicology*, *14*(1), 33–41. https://doi.org/10.1002/(sici)1099-0461(2000)14:1<33::aid-jbt5>3.3.co;2-p

Batool, Z., Agha, F., Ahmad, S., Liaquat, L., Tabassum, S., Khaliq, S., Anis, L., Sajid, I., Emad, S., Perveen, T., & Haider, S. (2017). Attenuation of cadmium-induced decline in spatial, habituation and recognition memory by long-term administration of almond and walnut supplementation: Role of cholinergic function. *Pakistan Journal of Pharmaceutical Sciences*, *30*(1), 273–279.

Beyersmann, D., & Hechtenberg, S. (1997). Cadmium, gene regulation, and cellular signalling in mammalian cells. *Toxicology and Applied Pharmacology*, *144*(2), 247–261. https://doi.org/10.1006/taap.1997.8125

Blum, J. L., Hoffman, C., Xiong, J. Q., & Zelikoff, J. T. (2010). Exposure of Pregnant Mice to Cadmium Oxide (CdO) Nanoparticles (NP) Poses a Risk to the Developing Offspring. *Biology of Reproduction*, *83* (Suppl_1), 295. https://doi.org/10.1093/biolreprod/83.s1.295

Blum, J. L., Xiong, J. Q., Hoffman, C., & Zelikoff, J. T. (2012). Cadmium associated with inhaled cadmium oxide nanoparticles impacts fetal and neonatal development and growth. *Toxicological Sciences*, *126*(2), 478–486. https://doi.org/10.1093/toxsci/kfs008

Brzóska, M. M., & Moniuszko-Jakoniuk, J. (2004). Low-level lifetime exposure to cadmium decreases skeletal mineralization and enhances bone loss in aged rats. *Bone*, *35*(5), 1180–1191. https://doi.org/10.1016/j.bone.2004.07.010

Byers, S. L., Wiles, M. V., Dunn, S. L., & Taft, R. A. (2012). Mouse estrous cycle identification tool and images. *PLoS ONE*, *7*(4). https://doi.org/10.1371/journal.pone.0035538

Calabrese, E. J. (2002). Part 1. The role of ROS in health disease: Part 2. Proposing a definition of hormesis. *Human and Experimental Toxicology*, *21*(2), 59. https://doi.org/10.1191/0960327102ht209oa

Careena, S., Sani, D., Tan, S. N., Lim, C. W., Hassan, S., Norhafizah, M., Kirby, B. P., Ideris, A., Stanslas, J., Bin Basri, H., & Lim, C. T. S. (2018). Effect of Edible Bird's Nest Extract on Lipopolysaccharide-Induced Impairment of Learning and Memory in Wistar Rats. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/9318789

Çavuşoğlu, K., Yapar, K., & Yalçin, E. (2009). Royal jelly (honey bee) is a potential antioxidant against cadmium-induced genotoxicity and oxidative stress in albino mice. *Journal of Medicinal Food*, *12*(6), 1286–1292. https://doi.org/10.1089/jmf.2008.0203

Chedrese, P., Piasek, M., & Henson, M. (2008). Cadmium as an Endocrine Disruptor in the Reproductive System. *Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry*, *6*(1), 27–35. https://doi.org/10.2174/187152206775528941

Cheng, Y., Zhang, J., Wu, T., Jiang, X., Jia, H., Qing, S., An, Q., Zhang, Y., & Su, J. (2019). Reproductive toxicity of acute Cd exposure in mouse: Resulting in oocyte defects and decreased female fertility. *Toxicology and Applied Pharmacology*, *379*. https://doi.org/10.1016/j.taap.2019.114684

Cheng, Yuyao, Zhang, J., Wu, T., Jiang, X., Jia, H., Qing, S., An, Q., Zhang, Y., & Su, J. (2019). Reproductive toxicity of acute Cd exposure in mouse: Resulting in oocyte defects and decreased female fertility. *Toxicology and Applied Pharmacology*, *379*(July), 114684. https://doi.org/10.1016/j.taap.2019.114684

Cheon, K. W., Lee, H. S., Parhar, I. S., & Kang, I. S. (2001). Expression of the second isoform of gonadotrophin-releasing hormone (GnRH-II) in human endometrium throughout the menstrual cycle. *Molecular Human Reproduction*, *7*(5), 447–452. https://doi.org/10.1093/molehr/7.5.447

Chi, B. Y., & Wang, C. H. E. (1921). *Proteins of Edible Birds ' Nests*. *5*.

Chua, K. H., Lee, T. H., Nagandran, K., Md Yahaya, N. H., Lee, C. T., Tjih, E. T. T., & Abdul Aziz, R. (2013). Edible Bird's nest extract as a chondro-protective agent for human chondrocytes isolated from osteoarthritic knee: In vitro study. *BMC Complementary and Alternative Medicine*, *13*(January). https://doi.org/10.1186/1472-6882-13-19

Chua, L. S., & Zukefl, S. N. (2016). A comprehnsive review of edible bird nests and swiftlet farming. *Journal of Integrative Medicine*, *14*(6), 415–428. https://doi.org/10.1016/S2095-4964(16)60282-0

Chua, L. S., & Zukefli, S. N. (2016). A comprehensive review on edible bird nests and swiftlet farming. *Journal of Integrative Medicine*, *14*(6), 415–428. https://doi.org/10.1016/S2095-4964(16)60282-0

Comp, J. (2009). Protective role of garlic against cadmium toxicity in rats: Clinicopa-thological and histopathological studies By. *Egypt. J. Comp. Path. & Clinic.*, *22*(3).

Cora, M. C., Kooistra, L., & Travlos, G. (2015). Vaginal Cytology of the Laboratory Rat and Mouse:Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. *Toxicologic Pathology*, *43*(6), 776–793. https://doi.org/10.1177/0192623315570339

D'Errico, J. N., Doherty, C., Fournier, S. B., Renkel, N., Kallontzi, S., Goedken, M., Fabris, L., Buckley, B., & Stapleton, P. A. (2019). Identification and quantification of gold engineered nanomaterials and impaired fluid transfer across the rat placenta via ex vivo perfusion. *Biomedicine and Pharmacotherapy*, fek*117*(May), 0–6. https://doi.org/10.1016/j.biopha.2019.109148

da Costa, C. S., de Oliveira, T. F., Freitas-Lima, L. C., Padilha, A. S., Krause, M., Carneiro, M. T. W. D., Salgado, B. S., & Graceli, J. B. (2020). Subacute cadmium exposure disrupts the hypothalamic-pituitary-gonadal axis, leading to polycystic ovarian syndrome and premature ovarian failure features in female rats. *Environmental Pollution*, *269*, 116154. https://doi.org/10.1016/j.envpol.2020.116154

da Silva Faria, T., de Bittencourt Brasil, F., Sampaio, F. J. B., & da Fonte Ramos, C. (2010). Effects of maternal undernutrition during lactation on estrogen and androgen receptor expressions in rat ovary at puberty. *Nutrition*, *26*(10), 993–999. https://doi.org/10.1016/j.nut.2009.09.027

Dai, Y., Cao, J., Wang, Y., Chen, Y., & Jiang, L. (2020). A comprehensive review of edible bird's nest. *Food Research International*, *November*, 109875. https://doi.org/10.1016/j.foodres.2020.109875

Dailiah Roopha, P., & Padmalatha, C. (2012). Effect of Herbal Preparation on Heavy Metal (Cadmium) Induced Antioxidant System in Female Wistar Rats. *Journal of Medical Toxicology*, *8*(2), 101–107. https://doi.org/10.1007/s13181-011-0194-y

Daud, N. 'Aliah, Sarbini, S. R., Babji, A. S., Mohamad Yusop, S., & Lim, S. J. (2019). Characterization of edible swiftlet's nest as a prebiotic ingredient using a simulated colon model. *Annals of Microbiology*, *69*(12), 1235–1246. https://doi.org/10.1007/s13213-019-01507-1

Daud, N. A., Yusop, S. M., Babji, A. S., Lim, S. J., Sarbini, S. R., & Yan, T. H. (2019). Edible Bird ' s Nest : Physicochemical Properties, Production, and Application of Bioactive Extracts and Glycopeptides. *Food Reviews International*, *00*(00), 1–20. https://doi.org/10.1080/87559129.2019.1696359

Debby, O. T. (1994). *Effect of Cadmium on Female Reproduction and Treatment Options*. https://doi.org/10.3923/rjog.2018.41.48

Dharmadasa, P., Kim, N., & Thunders, M. (2017). Maternal cadmium exposure and impact on foetal gene expression through methylation changes. *Food and Chemical Toxicology*, *109*, 714–720. https://doi.org/10.1016/j.fct.2017.09.002

Drummond, A. E. (2006). The role of steroids in follicular growth. *Reproductive Biology and Endocrinology*, *4*, 1–11. https://doi.org/10.1186/1477-7827-4-16

Epidemic, W. R. O. T. G. T. (2017). *Tobacco use kills more than 7 million people each year*.

Espart, A., Artime, S., Tort-Nasarre, G., & Yara-Varón, E. (2018). Cadmium exposure during pregnancy and lactation: materno-fetal and newborn repercussions of Cd(ii), and Cd-metallothionein complexes. *Metallomics*, *10*(10), 1359–1367. https://doi.org/10.1039/c8mt00174j

European Food Safety Authority. (2016). *Cadmium Dietary Exposure in the European Population*, *10*(1), 2551. https://doi.org/10.2903/j.efsa.2012.2551

Fang, J., Xie, S., Chen, Z., Wang, F., Chen, K., Zuo, Z., Cui, H., Guo, H., Ouyang, P., Chen, Z., Huang, C., Liu, W., & Geng, Y. (2021). Protective Effect of Vitamin E on Cadmium-Induced Renal Oxidative Damage and Apoptosis in Rats. *Biological Trace Element Research*. https://doi.org/10.1007/s12011-021-02606-4

Findlay, J. K., Kerr, J. B., Britt, K., Liew, S. H., Simpson, E. R., Rosairo, D., & Drummond, A. (2009). Ovarian physiology : follicle development , oocyte and hormone relationships. *Animal Reproduction / Colegio Brasileiro de*

Reproducao Animal, *6*(1), 16–19.

Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: A review with recent updates. *Interdisciplinary Toxicology*, *5*(2), 47–58. https://doi.org/10.2478/v10102-012-0009-2

Fondacci, C., Alsat, E., Gabriel, R., Blot, P., Nessmann, C., & Evain-Brion, D. (1994). Alterations of human placental epidermal growth factor receptor in intrauterine growth retardation. *Journal of Clinical Investigation*, *93*(3), 1149–1155. https://doi.org/10.1172/JCI117067

Fowles, J., Barreau, T., & Wu, N. (2020). Cancer and non-cancer risk concerns from metals in electronic cigarette liquids and aerosols. *International Journal of Environmental Research and Public Health*, *17*(6). https://doi.org/10.3390/ijerph17062146

Geng, H. X., & Wang, L. (2019). Cadmium: Toxic effects on placental and embryonic development. *Environmental Toxicology and Pharmacology*, *67*(2), 102–107. https://doi.org/10.1016/j.etap.2019.02.006

Giaginis, C., Gatzidou, E., & Theocharis, S. (2006). DNA repair systems as targets of cadmium toxicity. *Toxicology and Applied Pharmacology*, *213*(3), 282–290. https://doi.org/10.1016/j.taap.2006.03.008

Godbole, G. B., Modi, D. N., & Puri, C. P. (2007). Regulation of homeobox A10 expression in the primate endometrium by progesterone and embryonic stimuli. *Reproduction*, *134*(3), 513–523. https://doi.org/10.1530/REP-07-0234

Goh, D. L. M., Chua, K. Y., Chew, F. T., Seow, T. K., Ou, K. L., Yi, F. C., & Lee, B. W. (2001). Immunochemical characterization of edible bird's nest allergens. *Journal of Allergy and Clinical Immunology*, *107*(6), 1082–1088. https://doi.org/10.1067/mai.2001.114342

Goldman, J. M., Murr, A. S., & Cooper, R. L. (2007). The Rodent Estrous Cycle. *Birth Defects Research*, *80*(2), 83–97. https://doi.org/10.1002/bdrb

Guo, C. T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., Kato, K., Hidari, K. I. P. J., Miyamoto, D., Suzuki, T., & Suzuki, Y. (2006). Edible bird's nest extract inhibits influenza virus infection. *Antiviral Research*, *70*(3), 140–146. https://doi.org/10.1016/j.antiviral.2006.02.005

Gurel, E., Caner, M., Bayraktar, L., Yilmazer, N., Dogruman, H., & Demirci, C. (2007). Effects of artichoke extract supplementation on gonads of cadmium-treated rats. *Biological Trace Element Research*, *119*(1), 51–59. https://doi.org/10.1007/s12011-007-0048-2

Haghani, A., Mehrbod, P., Safi, N., Aminuddin, N. A., Bahadoran, A., Omar, A. R., & Ideris, A. (2016). In vitro and in vivo mechanism of immunomodulatory and antiviral activity of Edible Bird's Nest (EBN) against influenza A virus (IAV)

infection. *Journal of Ethnopharmacology*, *185*, 327–340. https://doi.org/10.1016/j.jep.2016.03.020

Hamzah, Z., Ibrahim, N. H., Sarojini, J., Hussin, K., Hashim, O., & Lee, B. (2013). Nutritional properties of edible bird nest. *Journal of Asian Scientific Research*, *3*(6), 600–607.

Hart, B. A., Potts, R. J., & Watkin, R. D. (2001). Cadmium adaptation in the lung - A double-edged sword? *Toxicology*, *160*(1–3), 65–70. https://doi.org/10.1016/S0300-483X(00)00436-4

Henson, M. C., & Chedrese, P. J. (2004). Endocrine Disruption by Cadmium, a Common Environmental Toxicant with Paradoxical Effects on Reproduction. *Experimental Biology and Medicine*, *229*(5), 383–392. https://doi.org/10.1177/153537020422900506

Hervé, M. A. J., Meduri, G., Petit, F. G., Domet, T. S., Lazennec, G., Mourah, S., & Perrot-Applanat, M. (2006). Regulation of the vascular endothelial growth factor (VEGF) receptor Flk-1/KDR by estradiol through VEGF in uterus. *Journal of Endocrinology*, *188*(1), 91–99. https://doi.org/10.1677/joe.1.06184

Hirshfield, A. N. (1997). Overview of ovarian follicular development: Considerations for the toxicologist. *Environmental and Molecular Mutagenesis*, *29*(1), 10–15. https://doi.org/10.1002/(SICI)1098-2280(1997)29:1<10::AID-EM2>3.0.CO;2-H

Höfer, N., Diel, P., Wittsiepe, J., Wilhelm, M., & Degen, G. H. (2009). Dose- and route-dependent hormonal activity of the metalloestrogen cadmium in the rat uterus. *Toxicology Letters*, *191*(2–3), 123–131. https://doi.org/10.1016/j.toxlet.2009.08.014

Hom, Y. K., Young, P., Wiesen, J. F., Miettinen, P. J., Derynck, R., Werb, Z., & Cunha, G. R. (1998). Uterine and vaginal organ growth requires epidermal growth factor receptor signaling from stroma. *Endocrinology*, *139*(3), 913–921. https://doi.org/10.1210/endo.139.3.5817

Hou, Z., Imam, M. U., Ismail, M., Azmi, N. H., Ismail, N., Ideris, A., & Mahmud, R. (2015). Lactoferrin and ovotransferrin contribute toward antioxidative effects of Edible Bird's Nest against hydrogen peroxide-induced oxidative stress in human SH-SY5Y cells. *Bioscience, Biotechnology and Biochemistry*, *79*(10), 1570–1578. https://doi.org/10.1080/09168451.2015.1050989

Hu, Q., Li, G., Yao, H., He, S., Li, H., Liu, S., Wu, Y., & Lai, X. (2016). Edible bird's nest enhances antioxidant capacity and increases lifespan in Drosophila Melanogaster. *Cellular and Molecular Biology*, *62*(4), 116–122. https://doi.org/10.14715/cmb/2016.62.4.20

Hussein, S. A., Ragab, O. A., & El-Eshmawy, M. A. (2014). Protective effect of green tea extract on cyclosporine a: Induced nephrotoxicity in rats. *Journal of*

Biological Sciences, *14*(4), 248–257. https://doi.org/10.3923/jbs.2014.248.257

Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, *54*(4), 287–293. https://doi.org/10.1016/j.ajme.2017.09.001

Ismahil, D. S. (2007). The Effect of Cadmium Chloride on Female Reproductive System of Wistar Rats. *Masters Thesis, University of Tehran, Iran*.

Ismail, & Aziz, F. M. (2017). The protective effect of omega-3 oil against the hepatotoxicity of cadmium chloride in adult and weanling rats. *AIP Conference Proceedings*, *1888*(September). https://doi.org/10.1063/1.5004304

Ismail, S. N. S., Salleh, F. H., Abidin, E. Z., Mahiddin, N. A. K., & Rangga, J. U. (2018). Cadmium (Cd) exposure among waste collector in Urban Area, Malaysia. *Malaysian Journal of Medicine and Health Sciences*, *14*(Cd), 72–80.

Iyengar, G., & Rapp, A. (2001). Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: Toxic trace elements in placenta and placenta as a biomarker for these elements. *Science of the Total Environment*, *280*, 221–238.

Jamalluddin, N. H., Tukiran, N. A., Ahmad Fadzillah, N., & Fathi, S. (2019a). Overview of edible bird's nests and their contemporary issues. *Food Control*, *104*(May), 247–255. https://doi.org/10.1016/j.foodcont.2019.04.042

Jamalluddin, N. H., Tukiran, N. A., Ahmad Fadzillah, N., & Fathi, S. (2019b). Overview of edible bird's nests and their contemporary issues. *Food Control*, *104*(April), 247–255. https://doi.org/10.1016/j.foodcont.2019.04.042

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*, 167–182. https://doi.org/10.1093/bmb/ldg032

Järup, L., & Åkesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology*, *238*(3), 201–208. https://doi.org/10.1016/j.taap.2009.04.020

Järup, L., Berglund, M., Elinder, C. G., Nordberg, G., & Vanter, M. (2015). - *a review Health effects of cadmium exposure literature and a risk estimate of the*. *24*(1), 1–51.

Johnson, M. D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G., Clarke, R., Sholler, P. F., Lirio, A. A., Foss, C., Reiter, R., Trock, B., Paik, S., & Martin, M. B. (2003). Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nature Medicine*, *9*(8), 1081–1084. https://doi.org/10.1038/nm902

Kathan, R. H., & Weeks, D. I. (1969). Structure studies of collocalia mucoid. I.

Carbohydrate and amino acid composition. *Archives of Biochemistry and Biophysics*, *134*(2), 572–576. https://doi.org/10.1016/0003-9861(69)90319-1

Khan, A., Ikram, M., Muhammad, T., Park, J., & Kim, M. O. (2019). Caffeine Modulates Cadmium-Induced Oxidative Stress, Neuroinflammation, and Cognitive Impairments by Regulating Nrf-2/HO-1 In Vivo and In Vitro. *Journal of Clinical Medicine*, *8*(5), 680. https://doi.org/10.3390/jcm8050680

Kim, B. H., Ju, W. S., Kim, J. S., Kim, S. U., Park, S. J., Ward, S. M., Lyu, J. H., & Choo, Y. K. (2020). Effects of gangliosides on spermatozoa, oocytes, and preimplantation embryos. *International Journal of Molecular Sciences*, *21*(1), 1–14. https://doi.org/10.3390/ijms21010106

Kim, J. J., Kim, Y. S., & Kumar, V. (2019). Heavy metal toxicity: An update of chelating therapeutic strategies. *Journal of Trace Elements in Medicine and Biology*, *54*(November 2018), 226–231. https://doi.org/10.1016/j.jtemb.2019.05.003

Klaassen, C. D., Liu, J., & Choudhuri, S. (1999). METALLOTHIONEIN: An Intracellular Protein to Protect Against Cadmium Toxicity. *Annual Review of Pharmacology and Toxicology*, *39*(1), 267–294. https://doi.org/10.1146/annurev.pharmtox.39.1.267

Klaassen, C. D., Liu, J., & Diwan, B. A. (2009a). Metallothionein protection of cadmium toxicity. *Toxicology and Applied Pharmacology*, *238*(3), 215–220. https://doi.org/10.1016/j.taap.2009.03.026

Klaassen, C. D., Liu, J., & Diwan, B. A. (2009b). Metallothionein protection of cadmium toxicity. *Toxicology and Applied Pharmacology*, *238*(3), 215–220. https://doi.org/10.1016/j.taap.2009.03.026

Knight, P. G., & Glister, C. (2003). Local roles of TGF-β superfamily members in the control of ovarian follicle development. *Animal Reproduction Science*, *78*(3–4), 165–183. https://doi.org/10.1016/S0378-4320(03)00089-7

Lacorte, L. M., Seiva, F. R. F., Rinaldi, J. C., Delella, F. K., Moroz, A., Sarobo, C., Godinho, A. F., Fávaro, W. J., Fernandes, A. A. H., & Felisbino, S. L. (2013). Caffeine reduces cadmium accumulation in the organism and enhances the levels of antioxidant protein expression in the epididymis. *Reproductive Toxicology*, *35*(1), 137–143. https://doi.org/10.1016/j.reprotox.2012.10.009

Lafuente, A. (2013). The hypothalamic-pituitary-gonadal axis is target of cadmium toxicity. An update of recent studies and potential therapeutic approaches. *Food and Chemical Toxicology*, *59*, 395–404. https://doi.org/10.1016/j.fct.2013.06.024

Large, M. J., Wetendorf, M., Lanz, R. B., Hartig, S. M., Creighton, C. J., Mancini, M. A., Kovanci, E., Lee, K. F., Threadgill, D. W., Lydon, J. P., Jeong, J. W., & DeMayo, F. J. (2014). The Epidermal Growth Factor Receptor Critically Regulates Endometrial Function during Early Pregnancy. *PLoS Genetics*,

10(6). https://doi.org/10.1371/journal.pgen.1004451

Lee. (2014). Smoking and Burden of Ill Health: A Review of the Malaysian Context. *International Journal of Collaborative Research on Internal Medicine & Public Health*, *6*(7), 190–198.

Lim, K. H., Teh, C. H., Pan, S., Ling, M. Y., Yusoff, M. F. M., Ghazali, S. M., Kee, C. C., Lim, K. K., Chong, K. H., & Lim, H. L. (2018). Prevalence and factors associated with smoking among adults in Malaysia: Findings from the National Health and Morbidity Survey (NHMS) 2015. *Tobacco Induced Diseases*, *16*(January), 1–12. https://doi.org/10.18332/tid/82190

Liu, Huang, H., Zhang, W., & Li, H. (2010). Cadmium-induced increase in uterine wet weight and its mechanism. *Birth Defects Research Part B - Developmental and Reproductive Toxicology*, *89*(1), 43–49. https://doi.org/10.1002/bdrb.20233

Liu, Y. (2000). Metallothionein-I/II Null Mice Are Sensitive to Chronic Oral Cadmium-Induced Nephrotoxicity. *Toxicological Sciences*, *57*(1), 167–176. https://doi.org/10.1093/toxsci/57.1.167

Luo, L. L., Huang, J., Fu, Y. C., Xu, J. J., & Qian, Y. S. (2008). Effects of tea polyphenols on ovarian development in rats. *Journal of Endocrinological Investigation*, *31*(12), 1110–1118. https://doi.org/10.1007/BF03345661

Lynes, M. A., Zaffuto, K., Unfricht, D. W., Marusov, G., Samson, J. S., & Yin, X. (2006). The physiological roles of extracellular metallothionein. *Experimental Biology and Medicine*, *231*(9), 1548–1554. https://doi.org/10.1177/153537020623100915

M. Brzóska, M., Borowska, S., & Tomczyk, M. (2016). Antioxidants as a Potential Preventive and Therapeutic Strategy for Cadmium. *Current Drug Targets*, *17*(12), 1350–1384. https://doi.org/10.2174/1389450116666150506114336

Ma, F., & Liu, D. (2012). Sketch of the edible bird's nest and its important bioactivities. *Food Research International*, *48*(2), 559–567. https://doi.org/10.1016/j.foodres.2012.06.001

Marcone, M. F. (2005). Characterization of the edible bird's nest the "Caviar of the East." *Food Research International*, *38*(10), 1125–1134. https://doi.org/10.1016/j.foodres.2005.02.008

Matés, J. M., Segura, J. A., Alonso, F. J., & Márquez, J. (2010). Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms. *Free Radical Biology and Medicine*, *49*(9), 1328–1341. https://doi.org/10.1016/j.freeradbiomed.2010.07.028

Matsukawa, N., MATSUMOTO, M., BUKAWA, W., CHIJI, H., NAKAYAMA, K., HARA, H., & TSUKAHARA, T. (2011). Improvement of Bone Strength and Dermal Thickness Due to Dietary Edible Bird's Nest Extract in Ovariectomized

Rats. *Bioscience, Biotechnology, and Biochemistry*, *75*(3), 590–592. https://doi.org/10.1271/bbb.100705

Matsukwa, N., Matsumoyo, M., Bukawa, W., Chihi, H., Nakayama, K., Hara, H., & Tsukahara, T. (2011). Improvement of bone strength and dermal thickness due to dietary edible bird's nest extract in ovariectomized rats. *Bioscience, Biotechnology and Biochemistry*, *75*(3), 590–592. https://doi.org/10.1271/bbb.100705

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. https://doi.org/10.3109/01480545.2013.878950

Mężyńska, M., & Brzóska, M. M. (2019). Review of polyphenol-rich products as potential protective and therapeutic factors against cadmium hepatotoxicity. *Journal of Applied Toxicology*, *39*(1), 117–145. https://doi.org/10.1002/jat.3709

Michels, U. (2003). *Atlas de música, 1*. *24*(5), 282. https://doi.org/10.1016/j.cellsig.2012.01.008.Reactive

Milnerowicz, H., Śliwińska-Mossoń, M., & Sobiech, K. A. (2017). The effect of ozone on the expression of metallothionein in tissues of rats chronically exposed to cadmium. *Environmental Toxicology and Pharmacology*, *52*, 27–37. https://doi.org/10.1016/j.etap.2017.03.010

Milton Prabu, S., Muthumani, M., & Shagirtha, K. (2012). Protective effect of Piper betle leaf extract against cadmium-induced oxidative stress and hepatic dysfunction in rats. *Saudi Journal of Biological Sciences*, *19*(2), 229–239. https://doi.org/10.1016/j.sjbs.2012.01.005

Mlynarcikova, A., Fickova, M., & Scsukova, S. (2005). Ovarian intrafollicular processes as a target for cigarette smoke components and selected environmental reproductive disruptors. *Endocrine Regulations*, *39*(1), 21–32.

Monsefi, M. (2013). *The Effects of Cadmium Pollution on Female Rat Reproductive System*. *1*(1), 2–6.

Nad, P., Massanyi, P., Skalicka, M., Korenekova, B., Cigankova, V., & Almasiova, V. (2007). The effect of cadmium in combination with zinc and selenium on ovarian structure in Japanese quails. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *42*(13), 2017–2022. https://doi.org/10.1080/10934520701629716

Nampoothiri, L. P., Agarwal, A., & Gupta, S. (2007). Effect of co-exposure to lead and cadmium on antioxidant status in rat ovarian granulose cells. *Archives of Toxicology*, *81*(3), 145–150. https://doi.org/10.1007/s00204-006-0133-x

Nash, A. D., Baca, M., Wright, C., & Scotney, P. D. (2006). The biology of vascular endothelial growth factor-B (VEGF-B). *Pulmonary Pharmacology and Therapeutics*, *19*(1), 61–69. https://doi.org/10.1016/j.pupt.2005.02.007

Nasiadek, M., Danilewicz, M., Klimczak, M., Stragierowicz, J., & Kilanowicz, A. (2019). Subchronic Exposure to Cadmium Causes Persistent Changes in the Reproductive System in Female Wistar Rats. *Oxidative Medicine and Cellular Longevity*, *2019*. https://doi.org/10.1155/2019/6490820

Nasiadek, M., Danilewicz, M., Sitarek, K., Ewa, Ś., Daragó, A., Stragierowicz, J., & Kilanowicz, A. (2018). *The effect of repeated cadmium oral exposure on the level of sex hormones , estrous cyclicity , and endometrium morphometry in female rats*. 28025–28038.

Nasiadek, M., Danilewicz, M., Sitarek, K., Świ\katkowska, E., Daragó, A., Stragierowicz, J., & Kilanowicz, A. (2018). The effect of repeated cadmium oral exposure on the level of sex hormones, estrous cyclicity, and endometrium morphometry in female rats. *Environmental Science and Pollution Research*, *25*(28), 28025–28038. https://doi.org/10.1007/s11356-018-2821-5

Nasiadek, M., Skrzypińska-Gawrysiak, M., Daragó, A., Zwierzyńska, E., & Kilanowicz, A. (2014). Involvement of oxidative stress in the mechanism of cadmium-induced toxicity on rat uterus. *Environmental Toxicology and Pharmacology*, *38*(2), 364–373. https://doi.org/10.1016/j.etap.2014.07.007

Nasiadek, M., Swiatkowska, E., Nowinska, A., Krawczyk, T., Wilczynski, J. R., & Sapota, A. (2011). The effect of cadmium on steroid hormones and their receptors in women with uterine myomas. *Archives of Environmental Contamination and Toxicology*, *60*(4), 734–741. https://doi.org/10.1007/s00244-010-9580-8

Niranjan, M. K., & Srivastava, R. (2019). Expression of estrogen receptor alpha in developing brain, ovary and shell gland of Gallus gallus domesticus: Impact of stress and estrogen. *Steroids*, *146*(May 2018), 21–33. https://doi.org/10.1016/j.steroids.2019.03.002

Nna, V. U., Usman, U. Z., Ofutet, E. O., & Owu, D. U. (2017). Quercetin exerts preventive, ameliorative and prophylactic effects on cadmium chloride - induced oxidative stress in the uterus and ovaries of female Wistar rats. *Food and Chemical Toxicology*, *102*, 143–155. https://doi.org/10.1016/j.fct.2017.02.010

Oguzturk, H., Ciftci, O., Aydin, M., Timurkaan, N., Beytur, A., & Yilmaz, F. (2012). *Ameliorative effects of curcumin against acute cadmium toxicity on male reproductive system in rats*. 243–249. https://doi.org/10.1111/j.1439-0272.2012.01273.x

Ospondpant, D., Phuagkhaopong, S., Suknuntha, K., Sangpairoj, K., Kasemsuk, T., Srimaroeng, C., & Vivithanaporn, P. (2019). Cadmium induces apoptotic

program imbalance and cell cycle inhibitor expression in cultured human astrocytes. *Environmental Toxicology and Pharmacology*, *65*(April 2018), 53–59. https://doi.org/10.1016/j.etap.2018.12.001

Othman, A. M., Abba, Y., Jesse, F. F. A., Ilyasu, Y. M., Saharee, A. A., Haron, A. W., Zamri-Saad, M., & Lila, M. A. M. (2016). Reproductive Pathological Changes Associated with Experimental Subchronic Corynebacterium pseudotuberculosis Infection in Nonpregnant Boer Does . *Journal of Pathogens*, *2016*, 1–7. https://doi.org/10.1155/2016/4624509

Pallares, P. (2009). *Original Article A new method for induction and synchronization of oestrus and fertile ovulations in mice by using exogenous hormones*. 295–299.

Pappas, R. S., Polzin, G. M., Zhang, L., Watson, C. H., Paschal, D. C., & Ashley, D. L. (2006). *Cadmium , lead , and thallium in mainstream tobacco smoke particulate*. *44*, 714–723. https://doi.org/10.1016/j.fct.2005.10.004

Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary Medicine International*, *2011*. https://doi.org/10.4061/2011/457327

Polykretis, P., Cencetti, F., Donati, C., Luchinat, E., & Banci, L. (2019). Cadmium effects on superoxide dismutase 1 in human cells revealed by NMR. *Redox Biology*, *21*(December 2018), 101102. https://doi.org/10.1016/j.redox.2019.101102

Prozialeck, W. C., & Edwards, J. R. (2012). Mechanisms of cadmium-induced proximal tubule injury: new insights with implications for biomonitoring and therapeutic interventions. *Journal of Pharmacology and Experimental Therapeutics*, *343*(1), 2–12. https://doi.org/10.1124/jpet.110.166769

Puppel, K., Kapusta, A., & Kuczyńska, B. (2015). The etiology of oxidative stress in the various species of animals, a review. *Journal of the Science of Food and Agriculture*, *95*(11), 2179–2184. https://doi.org/10.1002/jsfa.7015

Quartuccio, M., Cristarella, S., Medica, P., Fazio, E., Mazzullo, G., Rifici, C., Liotta, L., & Satué, K. (2020). Endometrial Cytology During the Different Phases of the Estrous Cycle in Jennies: New Evidences. *Animals*, *10*(6), 1062. https://doi.org/10.3390/ani10061062

Quek, M. C., Chin, N. L., Yusof, Y. A., Law, C. L., & Tan, S. W. (2018a). Characterization of edible bird's nest of different production, species and geographical origins using nutritional composition, physicochemical properties and antioxidant activities. *Food Research International*, *109*(September 2017), 35–43. https://doi.org/10.1016/j.foodres.2018.03.078

Quek, M. C., Chin, N. L., Yusof, Y. A., Law, C. L., & Tan, S. W. (2018b). Characterization of edible bird's nest of different production, species and geographical origins using nutritional composition, physicochemical properties

and antioxidant activities. *Food Research International*, *109*(September 2017), 35–43. https://doi.org/10.1016/j.foodres.2018.03.078

Ren, Y., Shao, W., Zuo, L., Zhao, W., Qin, H., Hua, Y., Lu, D., Mi, C., Zeng, S., & Zu, L. (2019). Mechanism of cadmium poisoning on testicular injury in mice. *Oncology Letters*, *18*(2), 1035–1042. https://doi.org/10.3892/ol.2019.10418

Renugadevi, J., & Prabu, S. M. (2010). Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. *Experimental and Toxicologic Pathology*, *62*(2), 171–181. https://doi.org/10.1016/j.etp.2009.03.010

Rikans, L. E., & Yamano, T. (2000). Mechanisms of cadmium-mediated acute hepatotoxicity. *Journal of Biochemical and Molecular Toxicology*, *14*(2), 110–117. https://doi.org/10.1002/(SICI)1099-0461(2000)14:2<110::AID-JBT7>3.0.CO;2-J

Roh, K. B., Lee, J., Kim, Y. S., Park, J., Kim, J. H., Lee, J., & Park, D. (2012). Mechanisms of edible bird's nest extract-induced proliferation of human adipose-derived stem cells. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/797520

Rotchell, J. M., Clarke, K. R., Newton, L. C., & Bird, D. J. (2001). Hepatic metallothionein as a biomarker for metal contamination: Age effects and seasonal variation in European flounders (Pleuronectes flesus) from the Severn Estuary and Bristol Channel. *Marine Environmental Research*, *52*(2), 151–171. https://doi.org/10.1016/S0141-1136(00)00270-1

Ruttkay-Nedecky, B., Nejdl, L., Gumulec, J., Zitka, O., Masarik, M., Eckschlager, T., Stiborova, M., Adam, V., & Kizek, R. (2013). The role of metallothionein in oxidative stress. *International Journal of Molecular Sciences*, *14*(3), 6044–6066. https://doi.org/10.3390/ijms14036044

Rzymski, P., Rzymski, P., Tomczyk, K., Niedzielski, P., Jakubowski, K., Poniedziałek, B., & Opala, T. (2014). Metal status in human endometrium: Relation to cigarette smoking and histological lesions. *Environmental Research*, *132*, 328–333. https://doi.org/10.1016/j.envres.2014.04.025

Rzymski, P., Tomczyk, K., Rzymski, P., Poniedziałek, B., Opala, T., & Wilczak, M. (2015). Impact of heavy metals on the female reproductive system. *Annals of Agricultural and Environmental Medicine*, *22*(2), 259–264. https://doi.org/10.5604/12321966.1152077

Saedi, S., Shirazi, M. R. J., Zamiri, M. J., Totonchi, M., Dadpasand, M., & Sedaghati, F. (2020). Impaired follicular development and endocrine disorders in female rats by prepubertal exposure to toxic doses of cadmium. *Toxicology and Industrial Health*, *36*(2), 63–75. https://doi.org/10.1177/0748233720912060

Saikia, P. J., Das, D., Mize, D., Das, M., & Sarma, H. N. (2017). Spatiotemporal expression of Vascular Endothelial Growth Factor-C in mice fetal-maternal tissues during periimplantation (D4–D7). *Middle East Fertility Society Journal*, *22*(2), 115–124. https://doi.org/10.1016/j.mefs.2016.10.001

Sajjad, S., Malik, H., Farooq, U., Rashid, F., Nasim, H., Tariq, S., & Rehman, S. (2014). Cadmium chloride toxicity revisited: Effect on certain andrological, endocrinological and biochemical parameters of adult male rabbits. *Physiological Research*, *63*(4), 505–512. https://doi.org/10.33549/physiolres.932641

Sakr, S. A., Bayomy, M. F., & El-Morsy, A. M. (2015). Rosemary extract ameliorates cadmium-induced histological changes and oxidative damage in the liver of albino rat. *The Journal of Basic & Applied Zoology*, *71*, 1–9. https://doi.org/10.1016/j.jobaz.2015.01.002

Samuel, J. B., Stanley, J. A., Princess, R. A., Shanthi, P., & Sebastian, M. S. (2011). Gestational Cadmium Exposure-Induced Ovotoxicity Delays Puberty through Oxidative Stress and Impaired Steroid Hormone Levels. *Journal of Medical Toxicology*, *7*(3), 195–204. https://doi.org/10.1007/s13181-011-0143-9

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.

Satarug, S., Garrett, S. H., Sens, M. A., & Sens, D. A. (2010). Cadmium, environmental exposure, and health outcomes. *Environmental Health Perspectives*, *118*(2), 182–190. https://doi.org/10.1289/ehp.0901234

Satarug, S., Haswell-Elkins, M. R., & Moore, M. R. (2000). Safe levels of cadmium intake to prevent renal toxicity in human subjects. *British Journal of Nutrition*, *84*(6), 791–802. https://doi.org/10.1017/s0007114500002403

Selvakumari, T., & Selvakumari, T. (2018). Female Reproductive System. *Essentials of Anatomy for Dental Students*, 611–611. https://doi.org/10.5005/jp/books/14250_57

Senger. (2005). *Pathways_to_Pregnancy_and_Parturition.pdf* (p. 370). Current conceptions.

Singh, N., Rani, P., Gupta, M., & Tandan, N. (2013). Role of Green Tea on Cadmium Toxicity on Haematological Profile of Albino Rats. *American Journal of Phytomedicine and Clinical Therapeutics*, *1*(5), 537–542.

Skipper, A., Sims, J. N., Yedjou, C. G., & Tchounwou, P. B. (2016). Cadmium chloride induces DNA damage and apoptosis of human liver carcinoma cells via oxidative stress. *International Journal of Environmental Research and Public Health*, *13*(1), 1–10. https://doi.org/10.3390/ijerph13010088

Smith, M. F. (1986). Recent Advances in Corpus Luteum Physiology. *Journal of Dairy Science*, *69*(3), 911–926. https://doi.org/10.3168/jds.S0022-0302(86)80481-7

Stepniak, J., & Karbownik-Lewinska, M. (2016). 17 β-estradiol prevents experimentally-induced oxidative damage to membrane lipids and nuclear DNA in porcine ovary. *Systems Biology in Reproductive Medicine*, *62*(1), 17–21. https://doi.org/10.3109/19396368.2015.1101510

Stohs, S. J., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine*, *18*(2), 321–336. https://doi.org/10.1016/0891-5849(94)00159-H

Suhy, D. A., Simon, K. D., Linzer, D. I. H., & O'Halloran, T. V. (1999). Metallothionein is part of a zinc-scavenging mechanism for cell survival under conditions of extreme zinc deprivation. *Journal of Biological Chemistry*, *274*(14), 9183–9192. https://doi.org/10.1074/jbc.274.14.9183

Tai, S. K., Koh, R. Y., Ng, K. Y., & Chye, S. M. (2017). *A Mini Review on Medicinal Effects of Edible Bird ' s Nest*. *June*. https://doi.org/10.15436/2475-6245.17.016

Tariba Lovaković, B. (2020). Cadmium, arsenic, and lead: elements affecting male reproductive health. *Current Opinion in Toxicology*, *19*(Figure 1), 7–14. https://doi.org/10.1016/j.cotox.2019.09.005

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy Metals Toxicity and the Environment. *Molecular, Clinical and Environmental Toxicology*, *101*, 133–164. https://doi.org/10.1007/978-3-7643-8340-4

Teotia, A., Lal, A., & Manu, P. (2013). Cellular mechanisms of cadmium-induced toxicity : A review International Journal of Environmental Health Research Cellular mechanisms of cadmium- induced toxicity : a review. *International Journal of Environmental Health Research*, *24*(4), 378–399. https://doi.org/10.1080/09603123.2013.835032

Thompson, J., & Bannigan, J. (2008). Cadmium : Toxic effects on the reproductive system and the embryo. *Reproductive Toxicology Journal*, *25*, 304–315. https://doi.org/10.1016/j.reprotox.2008.02.001

Trinchella, F., Riggio, M., Filosa, S., Volpe, M. G., Parisi, E., & Scudiero, R. (2006). Cadmium distribution and metallothionein expression in lizard tissues following acute and chronic cadmium intoxication. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *144*(3), 272–278. https://doi.org/10.1016/j.cbpc.2006.09.004

Turner, A. (2019). Cadmium pigments in consumer products and their health risks. *Science of the Total Environment*, *657*, 1409–1418. https://doi.org/10.1016/j.scitotenv.2018.12.096

van der Wall, E. E. (2010). Increasing recognition of NHJ: A first-time impact factor of 1.4! *Netherlands Heart Journal*, *18*(9), 399. https://doi.org/10.1007/BF03091804

Vardhan, K. H., Kumar, P. S., & Panda, R. C. (2019). A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. *Journal of Molecular Liquids*, *290*, 111197. https://doi.org/10.1016/j.molliq.2019.111197

Varga, B., Zsolnai, B., Paksy, K., Náray, M., & Ungváry, G. (1993). Age dependent accumulation of cadmium in the human ovary. *Reproductive Toxicology*, *7*(3), 225–228. https://doi.org/10.1016/0890-6238(93)90228-Y

Vignesh, K. S., & Deepe, G. S. (2017). Metallothioneins: Emerging modulators in immunity and infection. *International Journal of Molecular Sciences*, *18*(10). https://doi.org/10.3390/ijms18102197

Waisberg, M., Joseph, P., Hale, B., & Beyersmann, D. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, *192*(2–3), 95–117. https://doi.org/10.1016/S0300-483X(03)00305-6

Wallin, M., Sallsten, G., Lundh, T., & Barregard, L. (2014). Low-level cadmium exposure and effects on kidney function. *Occupational and Environmental Medicine*, *71*(12), 848–854. https://doi.org/10.1136/oemed-2014-102279

Wan, X., Zhu, J., Zhu, Y., Zhu, Y., Ma, X., Zheng, Y., Wang, F., Liu, Z., & Zhang, T. (2010). *Rat ovarian follicle bioassay reveals adverse effects of cadmium chloride (CdCl 2) exposure on follicle development and oocyte maturation*. https://doi.org/10.1177/0748233710375949

Wang, Cui, X., Cheng, H., Chen, F., Wang, J., Zhao, X., Lin, C., & Pu, X. (2015). A review of soil cadmium contamination in China including a health risk assessment. *Environmental Science and Pollution Research*, *22*(21), 16441–16452. https://doi.org/10.1007/s11356-015-5273-1

Wang, H., Liu, Z., Jia, X., Chen, H., & Tan, Y. (2014). Endocrine Disruption of Cadmium in Rats Using the OECD Enhanced TG 407 Test System. *Biomedical and Environmental Sciences : BES*, *27*, 950–959. https://doi.org/10.3967/bes2014.135

Wang, S., Ren, X., Hu, X., Zhou, L., Zhang, C., & Zhang, M. (2019). Cadmium-induced apoptosis through reactive oxygen species-mediated mitochondrial oxidative stress and the JNK signaling pathway in TM3 cells, a model of mouse Leydig cells. *Toxicology and Applied Pharmacology*, *368*(February), 37–48. https://doi.org/10.1016/j.taap.2019.02.012

Wang, Y., Wang, X., Wang, Y., Fan, R., Qiu, C., Zhong, S., Wei, L., & Luo, D. (2015). Effect of cadmium on cellular ultrastructure in mouse ovary. *Ultrastructural Pathology*, *39*(5), 324–328. https://doi.org/10.3109/01913123.2015.1027436

WHO. (2010). Exposure to cadmium: a major public health concern. *World Health Organization, Geneva, Switzerland*.

Williams, M., Bozhilov, K., Ghai, S., & Talbot, P. (2017). Elements including metals in the atomizer and aerosol of disposable electronic cigarettes and electronic hookahs. *PLoS ONE*, *12*(4), 1–24. https://doi.org/10.1371/journal.pone.0175430

Yang, J. M., Arnush, M., Chen, Q. Y., Wu, X. D., Pang, B., & Jiang, X. Z. (2003). Cadmium-induced damage to primary cultures of rat Leydig cells. *Reproductive Toxicology*, *17*(5), 553–560. https://doi.org/10.1016/S0890-6238(03)00100-X

Yew, M. Y. en., Koh, R. Y. ia., Chye, S. M. o., Othman, I., & Ng, K. Y. e. (2014). Edible bird's nest ameliorates oxidative stress-induced apoptosis in SH-SY5Y human neuroblastoma cells. *BMC Complementary and Alternative Medicine*, *14*, 391. https://doi.org/10.1186/1472-6882-14-391

Yida, Z., Imam, M. U., Ismail, M., Hou, Z., & Abdullah, M. A. (2015). Edible Bird ' s Nest attenuates high fat diet-induced oxidative stress and inflammation via regulation of hepatic antioxidant and inflammatory genes. *BMC Complementary and Alternative Medicine*, 1–7. https://doi.org/10.1186/s12906-015-0843-9

Yue, Z. P., Yang, Z. M., Li, S. J., Wang, H. Bin, & Harper, M. J. K. (2000). Epidermal growth factor family in rhesus monkey uterus during the menstrual cycle and early pregnancy. *Molecular Reproduction and Development*, *55*(2), 164–174. https://doi.org/10.1002/(SICI)1098-2795(200002)55:2<164::AID-MRD5>3.0.CO;2-D

Zhang, D., Liu, J., Gao, J., Shahzad, M., Han, Z., Wang, Z., Li, J., & Sjo, H. (2014). *Zinc Supplementation Protects against Cadmium Accumulation and Cytotoxicity in Madin-Darby Bovine Kidney Cells*. *9*(8), 1–10. https://doi.org/10.1371/journal.pone.0103427

Zhang, & Reynolds. (2019). Cadmium exposure in living organisms: A short review. *Science of the Total Environment*, *678*, 761–767. https://doi.org/10.1016/j.scitotenv.2019.04.395

Zhang, T., Gao, X., Luo, X., Li, L., Ma, M., Zhu, Y., Zhao, L., & Li, R. (2019). The effects of long-term exposure to low doses of cadmium on the health of the next generation of mice. *Chemico-Biological Interactions*, *312*(September), 108792. https://doi.org/10.1016/j.cbi.2019.108792

Zhang, W., & Jia, H. (2007). Effect and mechanism of cadmium on the progesterone synthesis of ovaries. *Toxicology*, *239*, 204–212. https://doi.org/10.1016/j.tox.2007.07.007

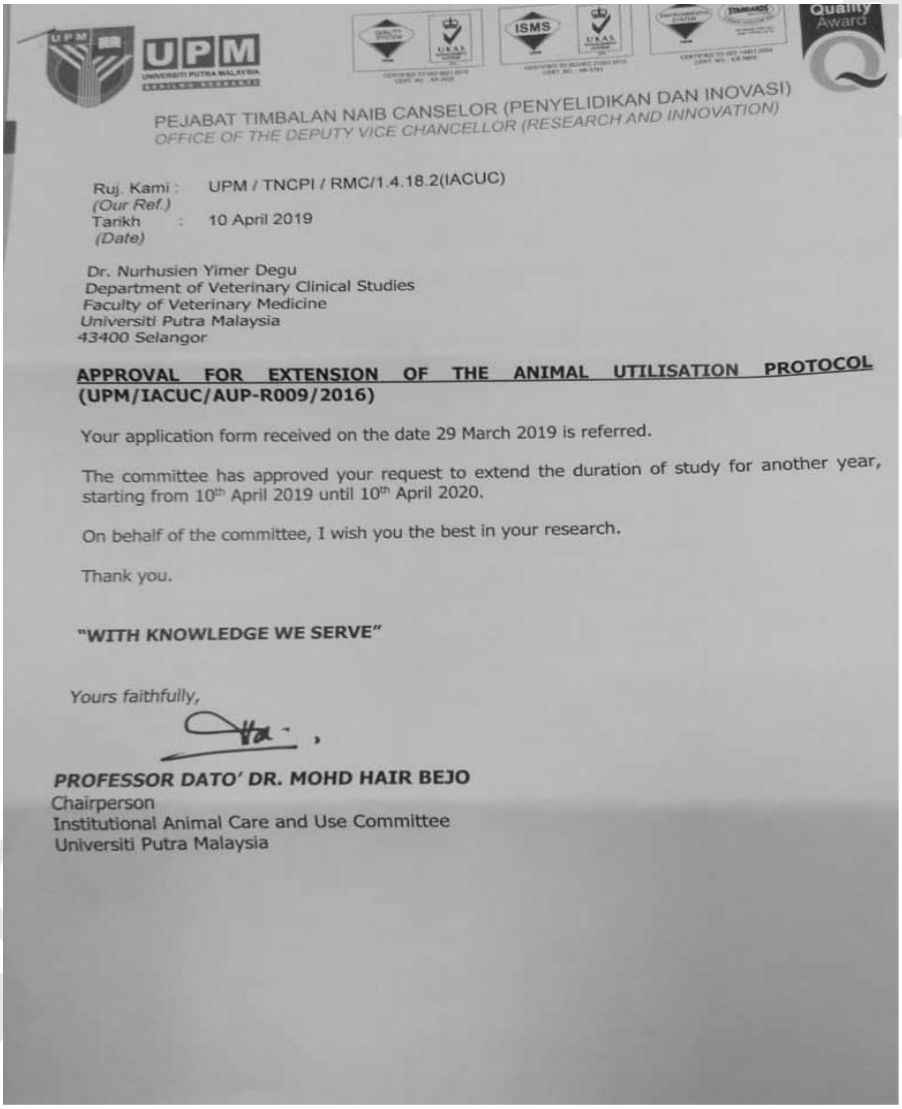
Zhiping, H., Imam, M. U., Ismail, M., Ismail, N., Yida, Z., Ideris, A., Sarega, N., & Mahmud, R. (2015). Effects of edible bird's nest on hippocampal and cortical neurodegeneration in ovariectomized rats. *Food and Function*, *6*(5), 1701–1711. https://doi.org/10.1039/c5fo00226e

Zucchetto A, Serraino D, Polesel J, Negri E, De Paoli A, Dal Maso L, Montella M, La Vecchia C, Franceschi S, Talamini R. Hormone-related factors and gynecological conditions in relation to endometrial cancer risk. Eur J Cancer Prev. 2009 Aug;18(4):316-21. doi: 10.1097/cej.0b013e328329d830. PMID: 19554665.

APPENDICES

Appendix A

Ethical Approval Letter, UPM



PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

Ruj. Kami : UPM / TNCPI / RMC/1.4.18.2(IACUC)
(Our Ref.)
Tarikh : 10 April 2019
(Date)

Dr. Nurhusien Yimer Degu
Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia
43400 Selangor

APPROVAL FOR EXTENSION OF THE ANIMAL UTILISATION PROTOCOL (UPM/IACUC/AUP-R009/2016)

Your application form received on the date 29 March 2019 is referred.

The committee has approved your request to extend the duration of study for another year, starting from 10th April 2019 until 10th April 2020.

On behalf of the committee, I wish you the best in your research.

Thank you.

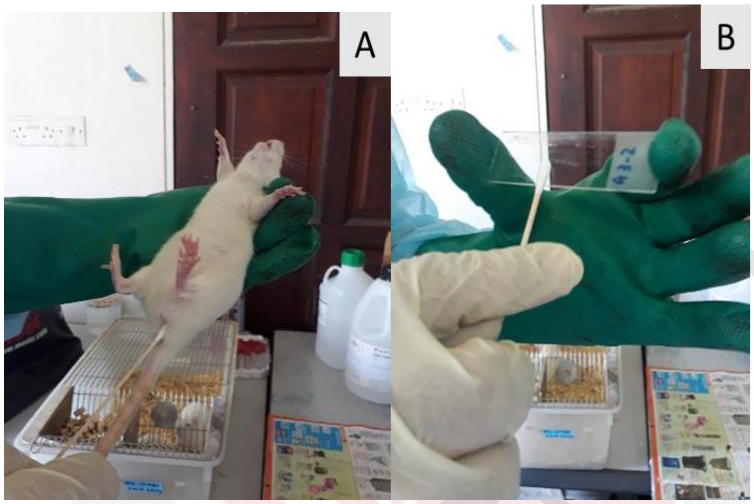
"WITH KNOWLEDGE WE SERVE"

Yours faithfully,

PROFESSOR DATO' DR. MOHD HAIR BEJO
Chairperson
Institutional Animal Care and Use Committee
Universiti Putra Malaysia

Appendix B

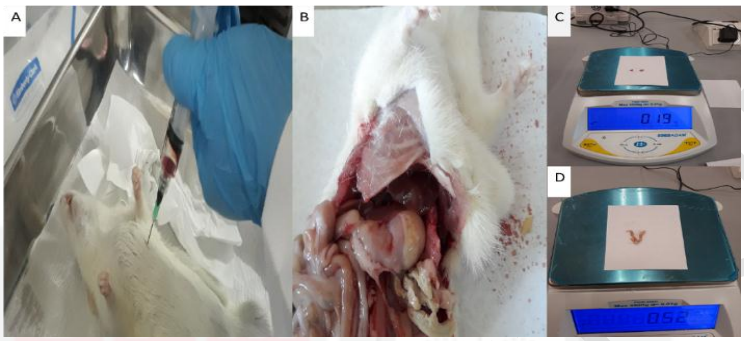
Vaginal Smears to observe estrus cyclicity



Vaginal swab sampling (A) and preparation of smear on a microscope slide (B)

Appendix C

Sacrificing rats under general anesthesia, blood collection, ovaries, and uterus harvesting



A: Blood collection from anesthetized rat via cardiac puncture B: Abdomen dissection of rat C: Weighing Ovaries D: Weighing Uterus

Appendix D

ELISA procedure for E2

Reagent preparation

For the preparation of the standard solution**,** added 1 ml of sample dilution buffer into one standard tube (labeled as zero tubes), keeping the tube at room temperature for 10 minutes and mix it thoroughly. Labeled 7 Eppendorf (EP) tubes with ½, ¼, 1/8, 1/16, 1/32, 1/64, and blank respectively. Added 0.3 ml of the sample dilution buffer into each tube. Added 0.3 ml of the above standard solution (from zero tube) into 1st tube and mix thoroughly. Transferred 0.3ml from 1st tube into 2nd tube and mixed thoroughly. Transferred 0.3 ml from 2nd tube into 3rd tube and mixed, and so on. Sample dilution was used for the blank control. For the preparation of the biotin-labeled Antibody working solution, calculated the required total volume of the working solution: 0.1ml/well x quantity of wells (Allowed 0.1-0.2 ml more than total volume). Diluted the biotin-detection antibody with an Antibody dilution buffer at 1:100 and mixed them thoroughly. HRP- streptavidin conjugate working solution was prepared by calculating required volume of solution. Diluted the HRP- streptavidin with dilution buffer at 1:100 and mixed thoroughly.

Assay procedure

Washed plate two times before adding standard, sample, and control (blank) wells. Set standard, test samples, control (blank) wells on the pre-coated plate, respectively, and then recorded their positions. Added 50ul of standard, blank, or sample per well, the blank was added with standard buffer solution. Immediately added 50ul Biotin-labeled Antibody working solution into each well. Covered with plate sealer. Incubated for 45 minutes at 37C. After incubation, washed the plates three times with wash buffer and let the wash buffer stay in the wells for 1 minute each time. After the last wash, removed any remaining wash buffer by aspirating or decanting. Then, added 100ul HRP working solution in each well. Covered it with a new Plate sealer. Incubated for 30 minutes at 3fC. After incubation washed plate five times with wash buffer. 90ul TMB substrate solution was added. Incubated 10-20 minutes at 37C. Then 50ul stop solution was added. The color turned yellow immediately. Read the optical density (OD) absorbance at 450nm in a microplate reader immediately after adding the stop solution.

ELISA procedure for P_4

Reagent Preparation

Brought all reagents to room temperature (18-25 C) before use. Prepared 750mL wash buffer by diluting 30mL of concentrated wash buffer with 720 mL of distill water. For preparation of Standard working solution centrifuged the standard at 10,000 for 1 min. 0.1 mL of reference standard and sample diluent was added, mixed it thoroughly with a pipette. Then made serial solutions as needed. Dilution method: Took 7 EP tubes, added 500ul of the working solution to the 1st tube and mixed thoroughly. Pipetted 500uL of the former tube to the latter one according to these steps. For the Antibody working

solution, calculated the required amount before the experiment(50ul/well). Diluted the 100X concentrated Biotinylated Detection Ab to 1X working solution with Biotinylated Detection Ab diluent. HRP conjugate was also prepared as per requirement (100uL each well).

Assay procedure

Added the standard working solution to the 1st two columns: Each concentration of the solution was added in duplicate to each well, side by side (50uL for each well). Samples were added to other wells (50uL each well), immediately added Antibody working solution to every well. Covered the plate with sealer. Incubated the plate for 45 min at 37C. After incubation decanted the solution from wells, added 350uL wash buffer in each well, soaked for 1-2 minutes and aspirated the wash buffer from wells. Added 100uL HRP conjugate working solution to each well and incubated for 30 minutes at 37C. After incubation decanted the solution from each well, repeated the wash process for five times. Then added 90uL of substrate reagent to each well and covered with a new plate sealer. Incubated for about 15 minutes at 37C and protected the plate from light. Later added 50uL of stop solution to each well. Determined the OD of each well by using a microplate reader at 450nm.

Appendix E

H&E staining procedure for microscopical examination

Method

- Collected sectioned (4 μm) tissues cut from paraffin-embedded blocks on clean glass slides.
- Dewaxed through graded alcohols followed by rinsing the slides into running water.
- Stained in Haematoxylin for 5 minutes.
- Dipped the slides 2-3times in 1% acid alcohol. Washed in running water until turned blue.
- Stained in eosin for 30 sec to 1 minute.
- Dehydrated through alcohol series and cleared in xylene.
- Mounted with a coverslip using DPX and left to dry.

Giemsa staining for Vaginal cytology

- Dipped the smear (3-4 dips) into pure methanol for fixation of the smear, left to air dry for 1 minute.
- Flooded the slide with 5% Giemsa stain solution for 10-20 minutes.
- Flushed with tap water and left to air dry.

Appendix F

Immunohistochemistry protocol for rat uterine and ovarian tissues

Mounting and Deparaffinization of Tissue Sections:

- Collected sectioned (4 μm) tissues cut from paraffin-embedded blocks on clean glass slides.
- Rehydrated through graded alcohols followed by rinsing the slides into running water.
- Stained in Haematoxylin for 5 minutes.
- Dipped the slides 2-3times in 1% acid alcohol. Washed in running water until turned blue.
- Stained in eosin for 30 sec to 1 minute.
- Dehydrated through alcohol series and cleared in xylene.

Target Retrieval (Heat-Induced Epitope Retrieval)

Prior to use, the product was diluted 1:10 with distilled water

(nine parts water) to make a working solution.

1. Deparaffinized and rehydrated tissue sections.
2. Immersed sections in suitable containers filled with diluted Target Retrieval Solution.
3. Performed antigen retrieval (10minutes in high power for microwave with minimum volume 500-700ml of antigen retrieval**.**
4. Decanted Target Retrieval Solution and rinsed sections 2 to 3 times with room temperature buffer

Procedural Notes

STEP 1 PEROXIDASE BLOCK

1. Tapped off excess wash buffer. Using a lintless tissue, carefully wiped around the specimen to remove any remaining liquid and to keep reagent within the prescribed area.
2. Applied enough Peroxidase Block to cover specimen.
3. Incubated for 10 minutes (±1) minutes.
4. Rinsed gently with distilled water or wash buffer from a wash bottle (do not focus flow directly on tissue) and place in a fresh buffer bath.

STEP 2 PROTEIN BLOCK

1. Tapped off excess buffer and wiped slides as before.
2. Applied enough protein block to cover specimen.
3. Incubated to 5 (±1) minutes.
4. Rinsed gently with buffer solution from a wash bottle.

STEP 3 PRIMARY ANTIBODY OR NEGATIVE CONTROL REAGENT

5. Tapped off excess buffer and wiped slides as before.
6. Applied enough optimally diluted primary antibody or negative control reagent to the covered specimen.
7. Incubated at room temperature for overnight at 4 degrees.
8. The next day, Rinsed gently with a buffer solution from a wash bottle and placed it in a fresh buffer bath.

STEP 4 PEROXIDASE LABELLED POLYMER

1. Tapped off excess buffer and wiped slides as before.
2. Applied enough Apply HRP-conjugate to cover specimen.
3. Incubated 15 (±1) minutes. Rinsed slides as in Step 2

STEP 5 DAB+ SUBSTRATE-CHROMOGEN

1. Wiped slides as before. Added 30 µl (1 drop) DAB Chromogen to 1.5 ml (50 drops) of DAB Substrate, mixed by swirling and applied to the tissue.
2. Applied enough prepared DAB+ substrate-chromogen to cover specimen.
3. Incubated for 10 (±1) minutes.
4. Rinsed gently with distilled water from a wash bottle.

STEP 6 HEMATOXYLIN COUNTERSTAIN (optional)

1. Immersed slides in a bath of hematoxylin. The length of incubation depends on the strength of hematoxylin used. Rinsed gently in a distilled water bath.
2. Rinse slides in a bath of distilled or deionized water for 2–5 minutes.

STEP 7 MOUNTING

3. Specimens were mounted and cover slipped with an aqueous-based mounting medium.

Appendix G

Sample preparation of ICP-MS

Steps

1. Uterus and ovary were weighed separately.
2. Transferred into Teflon vessel chamber
3. 8 ml Nitric acid (65%) was added in the Teflon vessel.
4. Vessels were tightly sealed.
5. Vessels transferred to Microwave digestor and kept for 1 hour.
6. After 1 hour cleaned digested solution was obtained, it was diluted up to 100mL with ultrapure water.

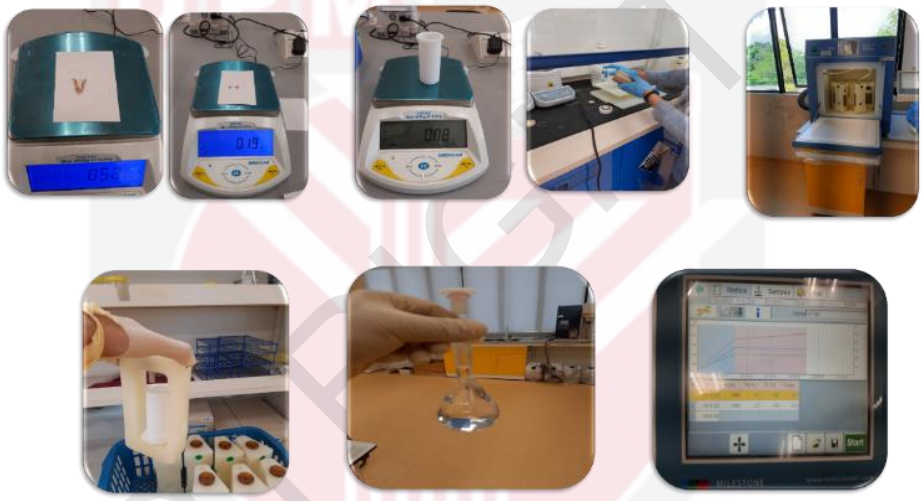


Figure: Steps involved in sample digestion

Appendix H

Histopathological lesion scoring

	Fields						
Organs/Scoring	1	2	3	4	5	6	Mean
Uterus							
Ovaries							

Histological scoring

Scoring Method	**0 (Normal)**	**1(Mild)**	**2 (Moderate)**	**3 (Severe)**
Histopathological changes	**00**	**<25**	**<50**	**>50**

BIODATA OF STUDENT

The student was born on 2nd May 1992 in the Quetta district of Balochistan province, Pakistan. He attended his primary and junior secondary education at Babar Model School from 1997 to 2008. He attained senior secondary education from Government degree college from 2008-2010. He gained admission for the degree of Veterinary Medicine at Lasbela University in 2011. He graduated in 2016 with the best grades in Clinical Medicine, Theriogenology, Veterinary Pathology, and Animal Nutrition.

He has been involved in Research activities from his final year in the university. Therefore, upon graduation, he was selected as a Research Officer at the Ministry of National Food Security, he gained diverse experience in agriculture and animal sciences research-related activities.

The student was awarded a master's Scholarship, he persuaded to Malaysia and gained admission in Theriogenology and cytogenetics with research focused on "Effect of Edible bird nest on ovarian and uterine functions of female rats subjected to Cd toxicity."

LIST OF PUBLICATIONS

Quddus, Nurhusien Yimer., M. M Noordin, Saadiya., Maria Amir, 2020. Antioxidant containing natural dietary products with ameliorating effect against Cadmium toxicity**.** ***Published in Pertanika Journal of Tropical Agricultural Sciences***

Quddus, Nurhusien Yimer., FFA Jesse, M. M Noordin., Mark W. H. Hiew, Maria Amir.,2020. Ameliorating Effects of Edible bird's Nest on Cadmium-induced Uterine Toxicity in Rats. ***Under review***

Quddus, Nurhusien Yimer., FFA Jesse, M. M Noordin., Mark W. H. Hiew, Maria Amir.,2020. Effects of cadmium on uterine functions and VEGF, EGF and EGFR expressions with respect to the protective effect of Edible bird nest in Sprague Dawley rats. ***Under review***

Quddus, Nurhusien Yimer., FFA Jesse, M. M Noordin., Mark W. H. Hiew, Maria Amir.,2020. Protective effect of Edible bird's nest on ovaries after acute cadmium exposure in cycling female rats. ***Drafted***

Conferences

Quddus, Nurhusien Yimer., FFA Jesse, M. M Noordin., Mark W. H. Hiew, Maria Amir.,2019. Ameliorating effect of EBN on ovarian function of cycling female rats subjected to cadmium toxicity. 31st Veterinary Association Malaysia Congress 2019, Bangi Resort, Malaysia. Oral presentation