Investigation of the profertility role of aqueousleaf extract of *Lawsonia Innermis* on adult female Wistar oviduct following Cadmium intoxication

ABSTRACT

Aim: Infertility, due to disruption of normal fertility environment by environmental pollutants and ubiquitous chemical, is a major call for public concern. For instance tubal factor infertility attributed to damage to the fallopian tube lining. Herbal remedy is an alternative to treat infertility due to their rich antioxidant potential. Henna *Lawsonia inermis* (Lythraceae) leaf is studied to report its role in protecting the tubal mucosa from environmental pollutants such as cadmium chloride to provide evidence on the effect of cadmium on tubal mucosa and potential antioxidant role of Henna (*Lawsonia inermis)* leaf extract (200mg/kg) to averts tubal factor infertility by evaluating changes in the histo-morphometric of tubal mucosal, biomarkers for oxidative stress and antioxidant enzyme.

Methodology: Twenty (20) adult female Wistar rats of four (4) groups (n=5) used were divided into control, cadmium (2mg/kg), 200mg/kg *Lawsonia inermis* leaf extract and 200 mg/kg *Lawsonia inermis* leaf extract treated cadmium induced infertility model. The excised fallopian tube were processed, stained using Haematoxylin and Eosin and Periodic Acid Schiff stain in addition to spectrophotometric analysis for serum level of Catalase (CAT) and Malondialdehyde (MDA).

Results: Results indicates marked disruption of mucosal lining and mucin granules of the tubes along with an elevation of MDA with a decline in CAT activity in cadmium induced infertility. This characterization was reversed in *Lawsonia inermis* leaf treated infertility model demonstrating gradual repair of the tubal mucosa lining attributed to elevation of antioxidant enzyme CAT that attenuated lipid peroxidation linked to oxidative tissue damage.

Conclusion:*Lawsonia inermis* (Henna) averts disruption of the tubal mucosal lining and loss of mucin granules by increasing antioxidant activity which averts lipid peroxidation mediated tubal epithelial mucosa damage.

Key words: *Lawsonia inermis,* infertility, mucin granules, malondialdehyde, catalase, cadmium, oxidative damage

1. INTRODUCTION

[Infertility was defined according toWHO (World Health Organization) as a disease of the reproductive system which makes a woman unable to carry pregnancy to full term [1]. Epidemiological studies show that the average prevalence of infertility in the world is 10%. [2] and Africa has the highest prevalence of infertility [3]). A number of heavy metal such as cadmium and lead are mostly studied for their potential in altering hormonal activity resulting in fertility problems [4]. They are described as toxins that acts by modulating endocrine activty in the female reproductive system, however there are limited studies on the mechansim involved through which these metals induced various types of infertility in females [4-6]). However a number of reports on cadmium induced in fertility to alteration in ovulation, hormonal imbalance, and fertilization [2.7]). Disruptions in female reproductive functions may lead to infertility, improper hormone production, estrous or menstrual cycle abnormalities, anovulation, and early reproductive ageing [8]. Traditional measures have been used to improve fertility rate [9,10]. Herbs have been proven to increase the fertility in females due to strong antioxidant complex or phytochemicals contained in them. Examples of such pro-fertility herbs include; red raspberry (stops excessive bleeding and strengthens uterus), trifoliumpratense (red clover blossoms) and Nettles (urticadioica) (maintains hormonal balance), motherwort and shwagandha [9]. *Lawsonia Inermis* (Henna), is a flowering plant and the sole species of the Lawsonia genus[11,12], this medicinal plant that has been widely used for herbal medicines globally because of its anti-inflammatory [13], ameliorative activity, antioxidant, antibacterial, among others [14,15]. The phytochemical screening reported on the dry leaves of *Lawsonia Inermis* has about 0.5-1.5% lawsone aside from phenolic glycosides alkaloids, anthocyanins, phenols, sterols, xanthoproteins, flavonoids, tannins, quinones, glycosides and saponins [15] Infertility, due to disruption of normal fertility environment by environmental pollutants and ubiquitous chemical, is a major call for public concern, that may contribute to reproductive disorders such as tubal factor infertility attributed to damage to the fallopian tube lining or ciliary activity that hinders eggs propulsion.Lawsonia inermis (Lythraceae) leaf is studied to report its role in protecting the tubal mucosa from cadmium induced tubal factor infertility by evlauating changes in tubal mucosa mucin granules, mucosal folds, antioxidant enzyme status and lipid peroxidation actvity.

2. MATERIAL AND METHODS

2.1 Experimental animals:

Twenty (20) adult female Wistar rats with average weight of about 150g procured from the National Veterinary Research Institution (NVRI), Vom, Jos Plateau State, Nigeria and housed in the animal folding facility of the Department of Anatomy Bingham University Karu, Nasarawa State were used for this study. They were housed in well aerated metallic cages and enable to acclimatized for two weeks, maintained at standard laboratory condition of 12-12h photoperiodicity, room temperature before the experiment commenced while be feed with water and pelleted rat feed (Vital feeds, Nasarawa) *ad libitum*. The animal care and use procedures in the research were performed in accordance with the Ethics Committee of the National Research Centre and the recommendations of the National Institutes of Health's Guide for Care and Use of Laboratory Animals [16].

2.2 Collection of the *Lawsonia Inermis Leaves:*The fresh *Lawsonia Inermis* leaves were harvested from Kastina-ala Local Government area in Benue State, Nigeria. The leaves were authenticated by a botanist in Ahmadu Bello University, Zaria, Kaduna State, Nigeria where vouncher Specimen number (900270) were catalogued in the herbarium.

2.3 Preparation of the *Lawsonia Inermis Leaves:* The collected ***Lawsonia Inermis Leaves*** were air- dried as described by Sulaiman *et al.,* [17] in a clean open space at room temperature until completely dried. The dried leaves were checked to remove any dirt before putting it in a blender (Philips Electric blender) to get its fine powdered form thereafter stored in a dried air-tight bottled and stored in clean cabinet [18].

2.4 Preparation of Aqueous Extract *Lawsonia Inermis* Leaves*:* The aqueous extract of ***Lawsonia Inermis*** leaves was extracted using the method described by Handa *et al.,* [19]. The extraction was done using maceration method which involved soaking its powdered form in water (solvent) for a period of time at room temperature for a period of time [19]. The processed intends to soften and break the plant's cell wall to release the soluble phytochemicals. Thereafter, the mixture was strained by filtration using a filter paper [18]. A total of 1800mg of the blended ***Lawsonia Inermis leaf powder*** was measured and soaked in distilled water for 48hrs then sieved and stored in a clean cool environment.

2.5 Chemical of Study and Dose of administration: Cadmium Chloride was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dose of study was taken to be 2mg/kg per body weight according to previous study [20,21] and given orally for a period of 4 days

2.6 Study dose of aqueous extract of *Lawsonia Inermis* Leaves: The study dose is 200mg/kg/day as demonstrated by Sravanthi et al., [22] and given orally for 14 days.

2.7 Experimental Animal grouping
Group A: the control group given water, feed *ad libtum*
Group B: the infertility model attributed to administration of oral 2mg/kg of Cadmium
Group C: given oral 200mg/kg/day of *Lawsonia Inermis* leaves
Group D: pretreated with oral 2mg/kg/day of Cadmium for 4 days then postreated for 14days using 200mg/kg/day *Lawsonia Inermis* leaves

2.8 Experimental design and protocol

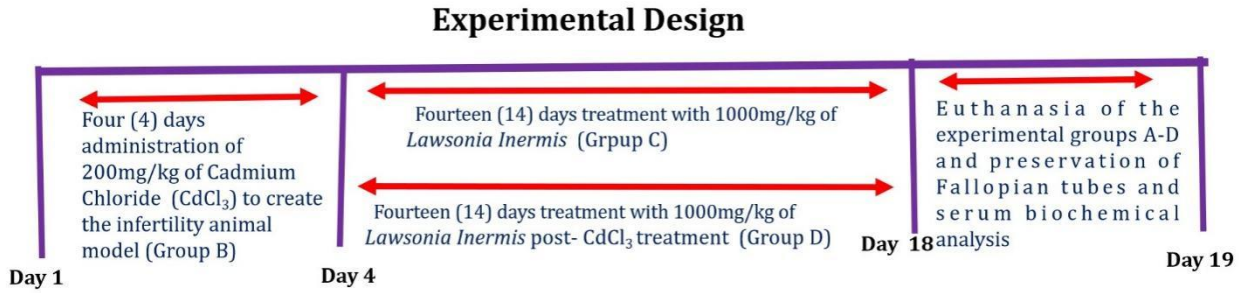

FiGURE 1: Illustartion of experimental design for Lawsonia inermis treatement
Duration of study: nine teen days two weeks (19 days)

2.9 Collection of Blood and preservation of Serum for biochemical analysis: Blood samples were collected through cardiac puncture using a 5ml syringe and blood collected transferred into labelled plain specimen bottles. Serum was obtained by centrifuging of blood at 3000 revolutions for 15 minutes The serum was aliquoted and transferred into labelled curvette placed on ice ready for spectrophotometric biochemical enzyme analysis using commercial test kits specific for each enzyme Catalase (CAT) and Malondialdehyde (MDA) uisng Catalase spectrophomteric test kit (ab83464) and Malondialdehyde ELISA Kit (ab287797) [23.24].

2.10 Experimental animal euthanasia and Fallopian tube collection: Twenty-four hours after the last dose administration the animals were euthanized, incision made in the lower abdominal region to expose the muscles and locate the fallopian tube. The excised fallopian tubes were rapidly transferred into 10% formol saline for preservation and automated tissue processing pending histological examination using Haematoxylin and Eosin (H and E) stain and Periodic Acid Schiff stain ([23,25].

2.11 Catalase (CAT) Analysis: In the UV range H_2O_2 shows a continual increase in absorption with decreasing wavelength. The difference in absorbance (ΔE_{240}) per unit time is measure of catalase activity. **Sample:** Blood serum. Spectral scan was taken on UV spectrophotometer at 520nm for 3minutes.

2,12 Estimation of Lipid Peroxidation: Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation.This method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde: an end product of lipid peroxide during peroxidation. Lipid peroxidation was estimated according to the method of Memudu et al., (2020) determined by measuring thiobarbituric acid-reactive species, producing a red-colored complex having a peak of absorbance at 532 nm [23.24].

2,13 Histological processing and staining of the fallopian tube: The tissues were processed according to method described by Memudu and Olutayo, 2021 method. The steps involved in tissue processing included fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning, sectioning, and staining. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol (50%, 70%, 90%, and absolute alcohol), then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58˚C. Three changes of molten paraffin wax at one- hour intervals were made, after which the tissues were embedded in wax and made into block of wax. Microtome whose

sectioning size knob was adjusted to six microns was then used to section the block, fixed on clean slides and later stained with haematoxylin and eosin (H and E), and Periodic Acid Schiff (PAS) [24,25].Histochemical staining was done to demonstrate mucin granules of the epithelial cells' basement membrane (basal lamina) using Periodic acid Schiff (PAS) reaction (enzyme digestion method) (**Memudu, and** Dongo 2023).

2.14 Tissue Photomicrography: Photomicrographs of sections of the renal cortices were obtained using a compound light microscope (Olympus Tokyo, Japan) connected to a digital microscopic camera (Amscope Inc., Irvine, CA, USA).

2.15 Statistical Analysis: All data were statistically evaluated using one – way ANOVA (Analysis of Variance) on SPSS/17.0 software (SPSS Inc, Chicago, USA) and the data were presented as mean ± standard Deviation (SD). Student's t-tests were used for all paired comparisons and one-way ANOVA was used for all multiple comparisons followed by the post hoc Tukey test. Statistics were significant when p-values were lower than 0.05 and significant effects are indicated by asterisks (*p < 0.05)

3. RESULTS

***3.1 Lawsonia inermis* leaf extract improves antioxidant enzyme- CAT level which in turns attenuates lipid peroxidation:** *Lawsonia inermis* leaf extract (C) caused a marked increase in antioxidant enzyme CAT level when compared with the control, cadmium treated and the *Lawsonia inermis* leaf extract treated cadmium induced infertility model at P<0.05 (C* vs A,B,and D). However when comparing role of *Lawsonia inermis* in the Cadmium induced infertility versus cadmium treated group, it was observed that there was a marked decline in CAT activity in cadmium treated group as compared to the increase in *Lawsonia inermis* treated Cadmium induced infertility. Lipid peroxidation occur due to disruption of the lipid membrane layer of the cells which is marked by elevation in MDA, in this study, Cadmium induced infertility (B) had an increase in MDA activity when compared to other study group at p<0.05, however this increase was attenuated following treatment with aqueous leaf extract of *Lawsonia inermis*.

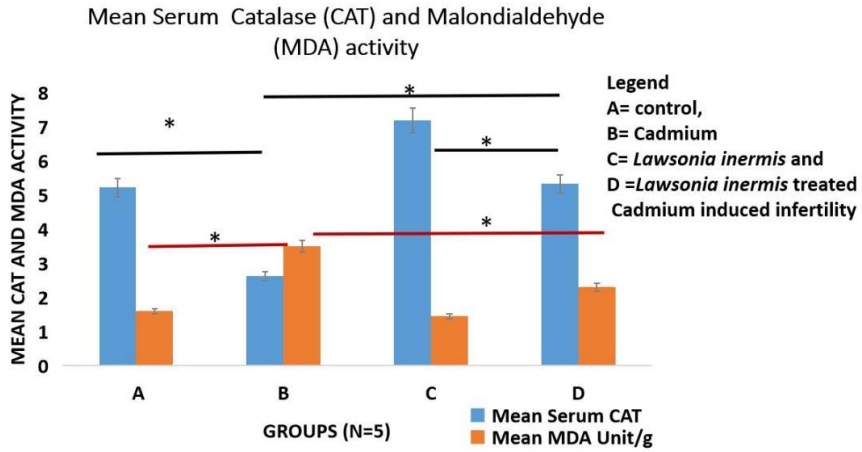

Figure 2: Graphical representation of serum Catalase (CAT) and Malondialdehyde (MDA) activity following administration of Cadmium and aqueous leaf extracts of *Lawsonia inermis* Legend A= control, B= Cadmium C- *Lawsonia inermis* and D=

Lawsonia inermis treated Cadmium induced infertility. CAT activity (A* vs B; D* vs B; C* vs B @ P<0.05). MDA activity (A* vs B; B* vs C; vs D @ p<0.05

3.2 Henna leaf extracts averts disruption of the mucosal folds and loss of mucin granules in tubal epithelial lining of the fallopian tube amidst cadmium assault: This study, demonstrates histomorphological and histochemical changes in the tubal epithelium following Cadmium intoxication and treatment with aqueous leaf extracts of *Lawsonia inermis*. Histological stain using Haematoxylin and Eosin (H and E) stain (Fig 3-A1-D1 at magnification of x4 while Fig 3 (A2-D2) at magnification of x40 shows the appearance of the mucosal simple columnar epithelium, underlying basal lamina, stroma cells and the basement membrane. The controls has well stained epithelium with presence of mucosal fold and normal mucosal epithelium when compares with the Cadmium treatment group have reduced mucosal fold, presence of sloughed off mucosal folds. aqueous leaf extracts of *Lawsonia inermis* treatment group demonstrates preservation and gradual restoration of perturbed mucosal epithelium and folds. Figure 4 (A-D) demonstrates Periodic Acid Schiff (PAS) stain for mucin granules or glycogen. The PAS stain in the control group depicts the reddish purple or magenta coloration for being positive to PAS reaction demonstrating presence of glycogen or mucin granules in the epithelial mucosa basal lamina. The cadmium treated mucosal epithelium appeared vaguely or poorly stained to PAS as seen in the yellow arrows in addition of pericellular spaces in the cells of the mucosal folds, This was not seen in the mucosal of the experimental animals given aqueous leaf extracts of *Lawsonia inermis.* The Cadmium induced and post treated with aqueous leaf extracts of *Lawsonia inermis* depicts presence of moderate reaction to PAS as compared to the cadmium treated group (B).

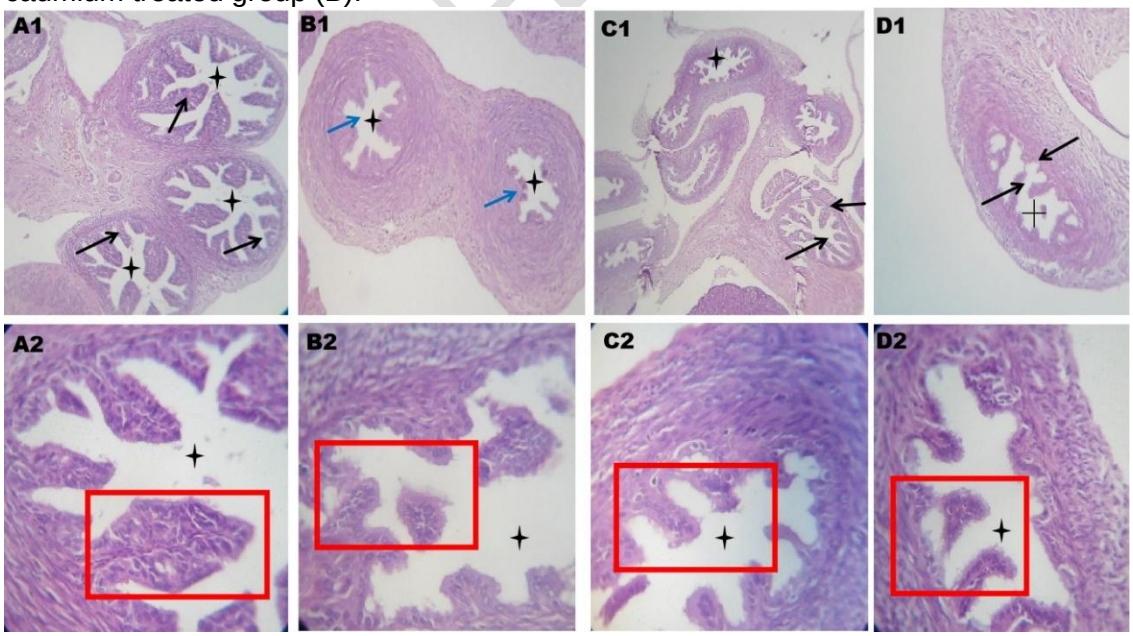

Figure 3: Photomicrograph of a horizontal section of the oviduct or fallopian tube of adult female Wistar rats stained using Haematoxylin and Eosin Stain. Magnification x 10 (A1-D1) and Magnification of x40 (A2-D2). Legend A= control, B= Cadmium C-Lawsonia inermis and D Lawsonia inermis treated Cadmium induced infertility. Dark

arrow and red box- mucosal fold, Blue arrows- ruputured mucosa folds, balck cross: tubal lumen,

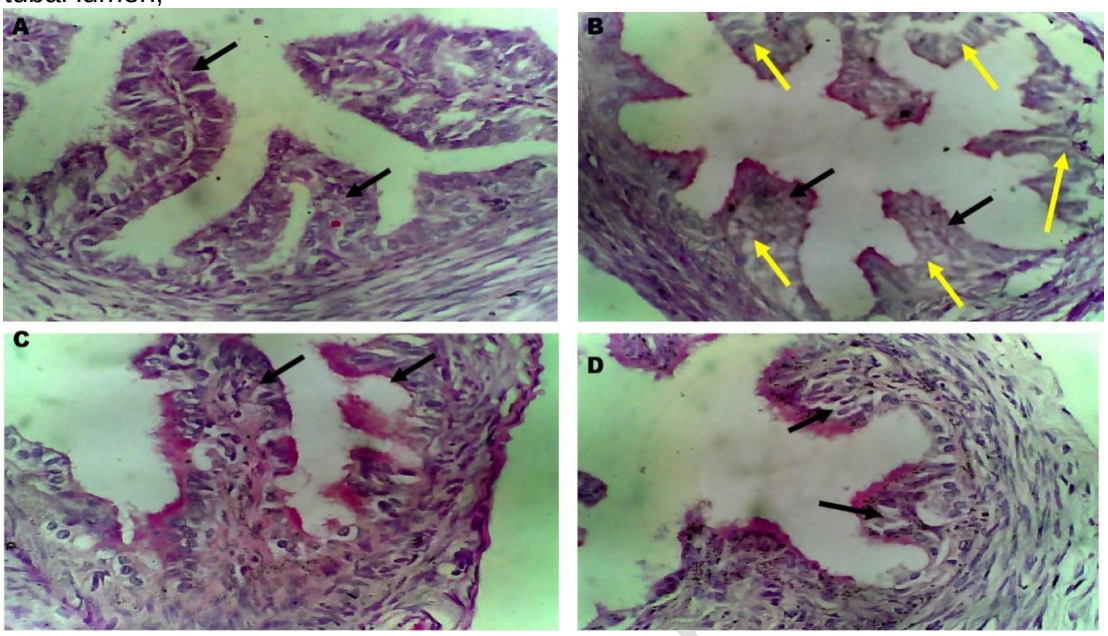

Figure 4: Photomicrograph of a horizontal section of the oviduct or fallopian tube of adult female Wistar rats stained using Periodic Acid Schiff stain Magnification of x40 Legend A= control, B= Cadmium C- Lawsonia inermis and D Lawsonia inermis treated Cadmium induced infertility. Dark arrow and red box- mucosal fold, Yellow arrows- poorly ruputured mucosa folds poorly stained for mucin granules with marked vacuolation in the epithelial fold columnar cells.

4. DISCUSSION

Infertility is a become global health issues defined as the inability to conceive after a year or more of regular unprotected intercourse by couples [26]. There are various factors in female infertility one of which is the tubal factor risk in female infertility which accounts for 30%-40% of a woman's fertility attributed to distruption in the normal mucosal lining of the fallopian tube as a result of inflammation [27]. Scientific studies to provide therapeutics against female infertility using herbal remedies have gained a lot of popularity over the years owing to their potent diverse phytochemicals such as like alkaloids, flavonoids, and essential oils with strong antioxidant propert [26]. The etiology of female fertility is complex as it involves various factor attributed to inflammation and oxidative tissue damage [28]. Cadmium has been reported to induced female infertility hence ,mostly used to model female infertility dince it has ability to distrupt endocrine activiities [29] by disrupting chemicals properties for hormone synthesis, secretion, and signaling [30]. Cadmium is an ubiquitous metal [31] that found in drinking water, food, soils, and air [32].. *Lawsonia inermis* is an important plant in ayurvedic or natural herbal medicines attributed to its phytoconstituents with antioxidant and anti-inflammatory activities to avert oxidative stress mediated tissue damage [33,34]. This present

study is to assess antioxidant and pro-fertility role of aqueous leaf extract of *Lawsonia inermis* on cadmium induces distruption of fallopian tube by evaluating changes in tubal mucosas, mucin granules, antioxidant enzyme status and lipid peroxidation actvity. Oxidative stress (OS), due to imbalance between the formation of reactive oxygen species (ROS) and antioxidant defenses is implicated in the pathophysiology of female infertility mediated by cadmium mediated bylipid peroxidation, DNA damage and mitochondrial dysfunction [35,36] Lipid peroxidation-induced damage reults in a weaken cell membrane, weaken function of the female reproductive cells mediated by proinflammatory cytokine formation that has also been induced by mitochondrial dysfunction resulting in a decline energy production and an increase in ROS mediating elevation of lipid proxidation[37,38]. The concentration of malondialdehyde (MDA) in the serum is an index of lipid peroxidation, determined spectrophotometrically indicated that Cadmium treatment caused an increase in MDA level. A number of reprot had hinted that Cadmium potentiate oxdiative stress and lipid peroxidation in tissue marked by ekevation of MDA activity [39-41]..This demonstrates that cadmium results in an increase in lipid peroxidation evident in the elevation of MDA activity when compared with the control group while declining the production of antioxidant CAT. The decline in antioxidant enzyme due to Cadmium intoxication is attributed to the depletion in antioxidant enzyme synthesized to combat the raging free radicals attchking the cells while distrupting cellular integrity to produce endogenous antioxidan enzyme. Cadmium mediated decline in CAT correlates with Ruslee et al., [42] and Maretta, and Marettová, [43]. Ruslee et al., [42] demonstrated a Cadmium induced oxidative stress with an increase in lipid peroxidation product, malondialdehyde (MDA) along with decreased levels of an antioxidant enzyme, catalase. . Aqueous leaf extract of *Lawsonia inermis* caused a marked increase in CAT activity while decreasing the production of MDA implying a decline in reactive oxygen species (ROS) release from tissues due to oxidative tissue damage in reponse to inflamamtion. The potential of *Lawsonia inermis* leaf to averts lipid perooxidation while improving antioxidant enzyme synthesis is attributed to its property as antioxidant, anti-inflamatory agents [44]. According to Massanyi et al.,[45] cadmium can perturb normal appearance of the oviduct lining. Cadmium can accumulate and interfer with normal progesterone secretion from the ovary which inturns influence oviduct epithelial cell to enable ease in transportation of oocyte and secretion in the lining that aid sperm fertilization of the oocytes [46]. Furthermore, cadmium potentiates distruption of of the columnar ciliated and secretory cells of the oviduct by distorting the lamina propria membrane integrity of the epithelium resulting in loss of thickness and mucosal folds [47,48]. This present study using histological stain and histochemical stain (Periodic Acid Schiff-PAS) fro mucin granules indicated that Cadmium cause a distortion in the oviduct epithelial lining with loss of connective tissue integrity and mucin granules. A study by Massányi, et al., [45,49] on rabbits oviduct shows that cadmium induced degeneration of the epithelium. Cadmium induced degeneration of fallopian tube epithelium via oxidative tissue damage is associated with distruption of nuclear chromatin of the epithelial cells [45]. we observed that cadmium caused a rupture of the mucosal folds in the oviducts, perturbed the stroma and mucin granules. *Lawsonia inermis* leaves treated infertility model had a marked regeneration of epithelial cells and protection of the mucosal folds. PAS is an histochemical stained

for glycogen, acidic and neutral mucins [23,50-51]. Cell-associated mucins shield the epithelial surface from pathogens through their extracellular domains and regulate intracellular signaling through their cytoplasmic regions (Lillehoj et al., [52].Glycosaminoglycan particles and mucins secreted by endometrial mucosal cells helps to protect microenironment of the fallopian tube for fertilization and oocyte survival [53]. Mucins are fluidiy gel on the surfaces of the secretory epithelium and helps to defend against harmful substances from the outside environment [54], aid hydration, lubrication, transport, and protection to the mucosa [55] and also stimulation of protective mechanisms, including promoting cell survival through their involvement in cell signaling regulating the processes of oocyte transport, implantation and maintaining pregrancy [56] Endometrial glycogen concentrations are correlated with fertility indicating that glycogen is an essential source of glucose for maintaining oocytes and implanatation [57]. Cadmium can depletes cellular level of glycogen [58,59]. This depletion of glycogen storage or mucin positive cells in the tubal mucosal treatd with cadmium support reports by Pawar, 59) however it is important to mention that glycogen are rapidly utilized in reproductive tract in normal condition. According to Li, et al., (60) glycogen metabolism and storage in the fallopian tube is interlinked through activation of the Wnt signal pathway where he oberved the presence of few cells in the epithelium and connective tissue with few glycogen stored in the cells. However the inflammed fallopian tissue had a dramatic increase in PAS cells in the tubal epithelium and muscularis. The *Lawsonia inermis* leaves treated group were positive to the presence of glycogen and mucin on the surfaces of the tubal mucosal epithelium. There is paucity of information on the effects of *Lawsonia inermis* leaves on mucin and glycogen granules in the fallopian tube. However from this present study one can document that PAS stain reveals that *Lawsonia inermis* leaves helps to moderately preserve glycogen store and ensure synethsis and release of mucin granules on the apical surface of tubal mucosal. This present study demonstrates that *Lawsonia inermis* leaves can averts cadmium induced infertility through its potent antioxidant and anti-inflamatory ability to boost CAT antioxidant level which in turn increase mobbing off of free radicals that alters tubal mucosal epithelium resulting in a decline in lipid peroxidation which results in regeneration of the epitthelial cells cytology characterized by moderate glycogen storage, secretion of mucin granules required to maintain the tubal mucosa milieu for oocytes transport and survival while mobbing off free radicals that can alter oocyte survival and transport.

5. CONCLUSION

Lawsonia inermis aqueous leaf extract demonstrates its profertility potential by reverting cadmium induced tubal mucosal lining dsitruption by increasing antioxidant activity, reducing lipid peroxidation mediate tubal epithelial mucosa damage and loss of mjucin or glycogen granules.

ETHICAL APPROVAL

THE ANIMAL CARE AND USE PROCEDURES IN THE RESEARCH WERE PERFORMED IN ACCORDANCE WITH THE ETHICS COMMITTEE OF THE

NATIONAL RESEARCH CENTRE AND THE RECOMMENDATIONS OF THE NATIONAL INSTITUTES OF HEALTH'S GUIDE FOR CARE AND USE OF LABORATORY ANIMALS

REFERENCES

1. Zegers-Hochschild F, Adamson, GD, Dyer, S, Racowsky C, de Mouzon J, Sokol, R, Rienzi L, Sunde A, Schmidt L, Cooke ID, Simpson, J, van der Poel. The International Glossary on Infertility and Fertility Care. Hum Reprod. 2017. 32(9):1786-1801.

2. Manouchehri A, Shokri S, Pirhadi M, Karimi M, Abbaszadeh S, Mirzaei G, Bahmani M...The Effects of Toxic Heavy Metals Lead, Cadmium and Copper on the Epidemiology of Male and Female Infertility. JBRA Assist Reprod. 2022. 926(4):627-630.

3. Direkvand Moghaddam A, Delpisheh A, Sayehmiri K. An Investigation of the Worldwide Prevalence of Infertility as a Systematic Review. Qom Univ Med Sci J. 2016. 10:76–87.

4. Lee S, Min JY, Min KB. Female Infertility Associated with Blood Lead and Cadmium Levels. Int J Environ Res Public Health. 2020 10;17(5):1794.

5. Banu, K. W., Senay, U., Atan and Melike, S. M. Effects of cadmium on female fertility parameters: an experimental study. journal Studies on Ethno-Medicine. 2017. 11(1).

6. Chen, W., Lau, S.W., Fan, Y., Wu, R.S. and Ge, W.. Juvenile exposure to bisphenol A promotes ovarian differentiation but suppresses its growth - Potential involvement of pituitary follicle-stimulating hormone. *Journal NCBI.* 2017.

7. Sengupta P., Banerjee R., Nath S., Das S., Banerjee S. Metals and female reproductive toxicity. Hum. Exp. Toxicol. 2015;34:679–697.

8. Rattan S., Brehm E., Gao L, Flaws, J.A. Di(2-ethylhexyl) phthalate exposure during prenatal development causes adverse transgenerational effects on female fertility in mice. Toxicological science. 2018. 163(2) :420-429

9. Akour A., Violet, K., Fatma, U., Afifi and Nailya, B. The use of medicinal herbs in gynecological and pregnancy-related disorders by Jordanian women: a review of folkloric practice vs. evidence-based pharmacology. Journal Pharmaceutical Biology. 2016. 54(9).

10. Kashani, L. and Akhondzadeh, S. . Female Infertility and Herbal Medicine. JMP (journal of medical plants),1 (61) :3-7

11. Bhuiyan, M.A., Islam, A., Ali, A. and Isalam, M.N. (2017). Colour and chemical constitution of natural dye henna and its application in textiles. Journal of cleaner production,167: 14-22.

12. Charoensup, R., Duangyod, T., Palanuvej, C. and Ruangrungsi, N. (2017).Pharmacognostic Specifications and Lawsone Content of Lawsonia inermis Leaves. Pharmacognosy Research, 9(1): 60–64.

13. :Borhani, M.S., Mohammad, R., Etemadifar, Z. and Karamifard, F. (2016). Assessment of the antimicrobial activity of Melissa officinalis and Lawsonia inermis extracts against some bacterial pathogens. Comparative Clinical Pathology, 25(1) :59–65

14. Chuku, L. C., Chinaka, N. C., & Damilola, D. (2020). Phytochemical Screening and Anti-inflammatory Properties of Henna Leaves (Lawsonia inermis). European Journal of Medicinal Plants, 31(18), 23–28. https://doi.org/10.9734/ejmp/2020/v31i1830340

15. :Supian FNA, Osman NI. Phytochemical and Pharmacological Activities of Natural Dye Plant, Lawsonia inermis L. (Henna). J Young Pharm. 2023;15(2):201-11.

16. National Institutes of Health's Guide for Care and Use of Laboratory Animals ::National Institutes of Health (NIH Publication No. 85–23, revised 1996)/National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 1986)

17. Sulaiman, S.F., Sajak, A.A.B., Ooi, K.L. and Supriatno, S.E.M. (2011). Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. *J Food Compos.Anal*, 24: 506-515

18. Azwanida, N.N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants*, 4: 196.

19. Handa, S.S., Khanuja. S.P.S., Longo, G. and Rakesh, D.D. (2008). Extraction Technologies for Medicinal and Aromatic Plants. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology.

20. Zhu Q, Li X, Ge RS. Toxicological Effects of Cadmium on Mammalian Testis. Front Genet. 2020 May 26;11:527. doi: 10.3389/fgene.2020.00527. PMID: 32528534; PMCID: PMC7265816.

21. Nofal, A. E., AboShabaan, H. S., Fayyad, R. M., Ereba, R. E., Omar, N. A., Elsharkawy, S. M., & Elberri, A. I. (2023). Immunostimulatory and anti-inflammatory impact of Fragaria ananassa methanol extract in a rat model of cadmium chloride-induced pulmonary toxicity. Frontiers in immunology, 14, 1297315. https://doi.org/10.3389/fimmu.2023.1297315

22. Sravanthi P, Kumar KM, Begum DT, Kumar SN, Ravindra reddy K, Khanhare RS (2011). Antiulcer activity of ethanolic extracts of Lawsonia inermis against cold stress induced ulceration in wistar rats. Journal of Pharmacy research. 4(6): 1872-1874.

23. Adejoke Elizabeth Memudu and Tayo Jane Oluwola (2021). The contraceptive potential of Carica papaya seed on oestrus cycle, progesterone, and histomorphology of the Utero-ovarian tissue of adult Wistar rat. JBRA Assisted Reproduction 2021;25(1):34-43 DOI: 10.5935/1518-0557.20200023

24. **Memudu, A.E and** Gambo A. Dongo (2023). A study to demonstrate the potential of Anabolic Androgen Steroid to activate oxidative tissue damage, nephrotoxicity and decline endogenous antioxidant system in renal tissue of Adult Wistar Rats.Toxicology Reports, 10: 320-326.https://doi.org/10.1016/j.toxrep.2023.02.010.

25. Bancroft and Gamble. (2008). Theory and practise of histological techniques. 6th edn. Churchill Livingstone, London

26. Chorosho SH, Malik N, Panesar G, Kumari P, Jangra S, Kaur R, Al-Ghamdi MS, Albishi TS, Chopra H, Singh R, Murthy HCA. Phytochemicals: Alternative for Infertility Treatment and Associated Conditions. Oxid Med Cell Longev. 2023 May 11;2023:1327562. doi: 10.1155/2023/1327562. PMID: 37215366; PMCID: PMC10195183.

27. Ambildhuke K, Pajai S, Chimegave A, Mundhada R, Kabra P. A Review of Tubal Factors Affecting Fertility and its Management. Cureus. 2022 Nov 1;14(11):e30990. doi: 10.7759/cureus.30990. PMID: 36475176; PMCID: PMC9717713.

28. :Cheng Y, Zhang J, Wu T, et al. Reproductive toxicity of acute Cd exposure in mouse: Resulting in oocyte defects and decreased female fertility. Toxicol Appl Pharmacol. 2019;379: 114684.

29. McClam, M., Liu, J., Fan, Y. et al. Associations between exposure to cadmium, lead, mercury and mixtures and women's infertility and long-term amenorrhea. Arch Public Health 81, 161 (2023). https://doi.org/10.1186/s13690-023-01172-6

30. Neblett MF, Curtis SW, Gerkowicz SA, et al. Examining reproductive health outcomes in females exposed to polychlorinated biphenyl and polybrominated biphenyl. Sci Rep. 2020;10(1):3314

31. : Bala R, Singh V, Rajender S, Singh K. Environment, lifestyle, and female infertility. Reprod Sci. 2021;28(3):617–38.

32. Okereafor U, Makhatha M, Mekuto L, Uche-Okereafor N, Sebola T, Mavumengwana V. Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. Int J Environ Res Public Health. 2020;17(7):2204.

33. :Manuja, A., Rathore, N., Choudhary, S., & Kumar, B. (2021). Phytochemical Screening, Cytotoxicity and Anti-inflammatory Activities of the Leaf Extracts from Lawsonia inermis of Indian Origin to Explore their Potential for Medicinal Uses. Medicinal chemistry (Shariqah (United Arab Emirates)), 17(6), 576–586. https://doi.org/10.2174/1573406416666200221101953

34. Khantamat, O., Dukaew, N., Karinchai, J., Chewonarin, T., Pitchakarn, P., & Temviriyanukul, P. (2021). Safety and bioactivity assessment of aqueous extract of Thai Henna (Lawsonia inermis Linn.) Leaf. Journal of toxicology and environmental health. Part A, 84(7), 298–312. https://doi.org/10.1080/15287394.2020.1866129

35. Kaltsas A, Zikopoulos A, Moustakli E, Zachariou A, Tsirka G, Tsiampali C, Palapela N, Sofikitis N, Dimitriadis F. The Silent Threat to Women's Fertility: Uncovering the Devastating Effects of Oxidative Stress. Antioxidants. 2023; 12(8):1490. https://doi.org/10.3390/antiox12081490

36. Ra, K.; Park, S.C.; Lee, B.C. Female Reproductive Aging and Oxidative Stress: Mesenchymal Stem Cell Conditioned Medium as a Promising Antioxidant. Int. J. Mol. Sci. 2023, 24, 5053

37. Su, L.J.; Zhang, J.H.; Gomez, H.; Murugan, R.; Hong, X.; Xu, D.; Jiang, F.; Peng, Z.Y. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxidative Med. Cell. Longev. 2019, 2019, 5080843.

38. Zhang, W.; Wu, F. Effects of adverse fertility-related factors on mitochondrial DNA in the oocyte: A comprehensive review. Reprod. Biol. Endocrinol. 2023, 21, 27

39. Dzobo, Kevin, & Naik, Yogeshkumar S.. (2013). Effect of selenium on cadmium-induced oxidative stress and esterase activity in rat organs. South African Journal of Science, 109(5-6), 01-08. Retrieved December 28, 2023, from http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0038-23532013000300012&lng=en&tlng=en.

40. Massányi, P., Massányi, M., Madeddu, R., Stawarz, R., & Lukáč, N. (2020). Effects of cadmium, lead, and mercury on the structure and function of reproductive organs. *Toxics*, *8*(4), 94.

41. Rotimi, D., Ojo, O. A., Emmanuel, B. A., Ojo, A. B., Elebiyo, T. C., Nwonuma, C. O., & Oluba, O. M. (2021). Protective impacts of gallic acid against cadmium-induced oxidative toxicity in the ovary of rats. *Comparative Clinical Pathology*, *30*, 453-460.

42. Ruslee S.S., Zaid S.S.M., Bakrin I.H., Goh Y.M., Mustapha N.M. Protective effect of Tualang honey against cadmium-induced morphological abnormalities and oxidative stress in the ovary of rats. BMC Complement. Med. Ther. 2020;20:1–11. doi: 10.1186/s12906-020-02960-1.

43. Maretta, M., & Marettová, E. (2022). Toxic Effects of Cadmium on the Female Reproductive Organs a Review. *Folia Veterinaria*, *66*(4), 56-66.

44. 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*, 6490820. https://doi.org/10.1155/2019/6490820

45. Massányi, P. and Lukáč, N. and Uhrín, V. and Toman, R. and Pivko, J. and Rafay, J. and Forgács, Zs. and Somosy, Z. (2007) Female reproductive toxicology of cadmium. Acta Biologica Hungarica, 58 (3). pp. 287-299. ISSN 0236-5383

46. Paksy K., Varga B., Náray M., Olajos F., Folly G. Altered ovarian progesterone secretion induced by cadmium fails to interfere with embryo transport in the oviduct of the rat. Reprod. Toxicol. 1992;6:77–83. doi: 10.1016/0890-6238(92)90024-N.

47. Sarkar A.K., Maitra S.K., Midya T. (1976) Histological, histochemical and biochemical effects of cadmium chloride in female koel (Eudynamys scolopacea) Acta Histochem. 1976;57:205–211. doi: 10.1016/S0065-1281(76)80049-9.

48. Sapmaz-Metin M., Topcu-Tarladacalisir Y., Kurt-Omurlu I., Weller B.K., Unsal-Atan S. A morphological study of uterine alterations in mice due to exposure to cadmium. Biotech. Histochem. 2017;92:264–273. doi: 10.1080/10520295.2017.1305500.

49. Massányi, P., Uhrín, V., Toman, R., Kovácik, J., Bíro, D. (1999). Histological changes in the oviduct of rabbits after administration of cadmium. Journal of Animal and Feed Sciences, 8(2), 255-261. https://doi.org/10.22358/jafs/68933/1999

50. Meyerholz DK, Stoltz DA, Gansemer ND, Ernst SE, Cook DP, Strub MD, et al. Lack of cystic fibrosis transmembrane conductance regulator disrupts fetal airway development in pigs. Lab Invest. 2018;98(6):825–838. doi: 10.1038/s41374-018-0026-7.

51. Abouelela, Y.S., Shaker, N.A., El-Shahat, K.H. et al. Anatomical, histological, and histochemical alterations in portio vaginalis uteri with an evaluation of the vaginal artery vascularity during the luteal and early pregnant stages in domestic buffaloes (Bubalus bubalis). BMC Vet Res 19, 260 (2023). https://doi.org/10.1186/s12917-023-03816-9

52. :Lillehoj EP, Kato K, Lu W, Kim KC. Cellular and molecular biology of airway mucins. International Review of Cell and Molecular Biology. 2013 ;303:139-202. DOI: 10.1016/b978-0-12-407697-6.00004-0. PMID: 23445810; PMCID: PMC5593132.

53. :Lu D, Hu W, Tian T, Wang M, Zhou M, Wu C. The Mechanism of Lipopolysaccharide's Effect on Secretion of Endometrial Mucins in Female Mice during Pregnancy. International Journal of Molecular Sciences. 2022; 23(17):9972. https://doi.org/10.3390/ijms23179972

54. :Dickinson, J.D.; Sweeter, J.M.; Staab, E.B.; Nelson, A.J.; Bailey, K.L.; Warren, K.J.; Jaramillo, A.M.; Dickey, B.F.; Poole, J.A. MyD88 controls airway epithelial Muc5ac expression during TLR activation conditions from agricultural organic dust exposure. Am. J. Physiol. Lung Cell. Mol. Physiol. 2019, 316, L334–L347.

55. Theodoropoulos, G.; Carraway, K.L. Molecular Signaling in the Regulation of Mucins. *J. Cell. Biochem.***2007**, *102*, 1103–1116

56. Zhou, M., Tian, T., & Wu, C. (2023). Mechanism Underlying the Regulation of Mucin Secretion in the Uterus during Pregnancy. International journal of molecular sciences, 24(21), 15896. https://doi.org/10.3390/ijms242115896

57. Dean M. (2019). Glycogen in the uterus and fallopian tubes is an important source of glucose during early pregnancy†. Biology of reproduction, 101(2), 297–305. https://doi.org/10.1093/biolre/ioz102

58. :Lowe-Jinde, L., Niimi, A.J. Short-term and long-term effects of cadmium on glycogen reserves and liver size in rainbow trout (Salmo gairdneri Richardson). Arch. Environ. Contam. Toxicol. 13, 759–764 (1984). https://doi.org/10.1007/BF01055940

59. Pawar, Rajkumar. (2021). EFFECT OF CADMIUM CHLORIDE ON GLYCOGEN LEVEL IN SOME ORGANS OF FRESHWATER BIVALVE, LAMELLIDENS MARGINALIS. International Journal of Biological Innovations. 3. 367-372. 10.46505/IJBI.2021.3218.

60. Li, Ping & Zhu, Wei-Jie & Ma, Zheng-Lai & Wang, Guang & Peng, Hui & Chen, Yao & Lee, Kenneth & Yang, Xuesong. (2013). Enhanced beta-catenin expression and inflammation are associated with human ectopic tubal pregnancy. Human reproduction (Oxford, England). 28. 10.1093/humrep/det246.