COMPARATIVE HISTOMORPHOLOGICAL AND HISTOSTEREOLOGICAL TERATOGENIC EFFECTS OF *IN-UTERO* EXPOSURE TO VARIED DOSES OF OMEPRAZOLE AND PANTOPRAZOLE ON FETAL KIDNEYS IN ALBINO RATS (*Rattus norvegicus)*

ANNE NJOKI NYAGA

MASTER OF SCIENCE

(Human Anatomy)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2024

Comparative Histomorphological and Histostereological Teratogenic Effects of *In-utero* Exposure to Varied Doses of Omeprazole and Pantoprazole on Fetal Kidneys in Albino Rats (*Rattus norvegicus)*

Anne Njoki Nyaga

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Human Anatomy of the Jomo Kenyatta University of Agriculture and Technology

2024

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature …….Date ……

Anne Njoki Nyaga

This thesis has been submitted for examination with our approval as University Supervisors

Signature …….Date ……

Dr. Joseph Kariuki Kweri, PhD

JKUAT, Kenya

Signature …….Date ……

Dr. Ann Wairimu Mwangi, PhD

JKUAT, Kenya

Signature …….Date ……

Dr. James Mwangi Kanyoni, PhD

JKUAT, Kenya

Signature …….Date ……

Dr. Caroline Chepngeno Sigei, PhD

JKUAT, Kenya

DEDICATION

I dedicate this thesis to Almighty God, and my parents; Alfred Nyaga and Winkate Kellen who have been my greatest pillar of support throughout the entire of time my study. My sisters and brothers, you have constantly supported me in so many ways, and dear Grace, you remained consistent, offering so much emotional support to me, thank you. To Irene, you have been such an incredible true friend and a source of inspiration to me.

ACKNOWLEDGEMENT

I wish to express my most sincere thanks to my supervisors Dr. Joseph Kweri, Dr. Ann Mwangi, Dr. James Mwangi Kanyoni, and Caroline Sigei for their continued guidance and support they accorded me in the whole process of developing this thesis. My Special thanks go to Dr. Joseph Kweri who has effortlessly dedicated his time and effort during my entire time of this study until now.

I would also want to thank Joshua Otieno for his support during the animal handling procedures and Pamela Emali for her assistance especially during the tissue processing.

Lastly, I wish to also acknowledge my classmates Jane Kuria, Joseph Wachira, Segut Jeniffer, and Cyrus Kamau, for their support during my study period.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AED	Animal Equivalent Dosage
ABC	ATP Binding Cassette
AKI	Acute Kidney Injury
ANC	Antenatal Clinic
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BCRP	Breast Cancer Resistance Protein
BW	Body Weight
CKD	Chronic Kidney Damage
ESRD	End Stage Renal Disease
FDA	Food and Drug Administration
GD	Gestation Day
GD_{15}	Gestation Day 15
HD	High Dose
HED	Human Equivalent Dose
H&E	Hematoxylin and Eosin
H^+K^+ATPases	Hydrogen and potassium ions Adenosine Triphosphat**e**
IARI	International Animal Research Institute
JKUAT	Jomo Kenyatta University of Agriculture and Technology
JPEG	Joint Photography Expert Group
PPI	Proton Pump Inhibitor

LD	Low Dose
MANOVA	Multiple Analysis of Variance
Mls	Milliliters
MD	Medium Dose
MDRI	Multidrug Resistance Protein -1
MG	Milligrams
OMEZ	Omeprazole
PAN	Pantoprazole
SAFARI	Small Animal Facility for Research and Innovation
SD	Standard Deviation
SPSS	Statistical Package of Social Science
TMI	Trimester one
TM2	Trimester two
TM3	Trimester three
µm	Micrometer
UON	University of Nairobi

DEFINITION OF OPERATIONAL TERMS

Chronic kidney disease This refers to the abnormalities of kidney structure and function for more than three months or the presence of structural or functional kidney damage.

Embryo lethality This referred to the death of the embryo and fetuses complain to the implantation site in the uterine horns

Fetal toxicity Increase in the fetal loss ad decreased fetal growth and development

Gastric Gavage This was a method of administering omeprazole and pantoprazole into the stomach through the mouth using a metallic tube in experimental animals.

Histo-stereology This is a three-dimensional measurement of microscopic structures important to obtain reliable quantitative data that enables calculation of volumes and volume ratio, in this study histostereology was used to determine the volume of the fetal kidney

Morphometry This referred to the quantitative description of geometric features of structures such as kidney tissue and cells.

Sickness In this study, this term referred to any abnormal behavior in albino rats that included red eyes, changes in skin, fur and mucous membrane, seizures, altered repertory effort, hunched posture, decreased feeding and diarrhea and bloody stools.

ABSTRACT

Omeprazole and pantoprazole are proton pump inhibitor medicines which are commonly used in management of gastric esophageal reflux disease during pregnancy. However, their teratogenic risk or their safety indexes on the developing fetal kidneys remains unclear. Further, whether their teratogenic effects are dose and time dependent is yet to be elucidated. The broad objective of this study therefore was to comparatively evaluate the histomorphological and the histostereological teratogenic effects of *in*-utero exposure to varied doses of omeprazole and pantoprazole on the developing kidneys in Albino rats (*Rattus norvegicus*). In carrying out this study, a post-test-only experimental study design with control was used. All the animal experimentation was carried out in the animal research facility at the University of Nairobi, while tissue processing for histology and stereological analysis was done at JKUAT, main campus. A Sample size of 30 Albino rats weighing between 190 to 230grams were used for each of the two study medicines. This sample size of 30 rats per group was determined by use of the resource equation for one-way Analysis of Variance method (ANOVA). The 30 Albino rats in each of the two study categories of omeprazole and pantoprazole were first randomly divided into two study groups of 3 rats control and 27 rats in treatment category. To evaluate whether the teratogenic effects of both medicines are dose dependent, the 27 rats in the experimental category were subdivided into three study sub groups of 9 rats each as follows; (i) 9 rats for low doses of omeprazole and pantoprazole group (2.07mg/kg/4.13mg/kg, respectively), (ii) 9 rats for medium doses of omeprazole and pantoprazole group (medium19.63mg/kg /13.43mg/kg, respectively), (iii) 9 rats for the high omeprazole and pantoprazole group (37.8mg/kg, /24.8mg/kg). To further evaluate whether the observed teratogenic effects are time dependent, the 9 rats in each of the three dose categories were further sub-divided into three subgroups of 3 rats each according to the trimesters of exposure as follows; (i) 3 rats for trimester one (ii) 3 rats for trimester two and (iii) 3 rats for trimester three. All the rats in both the control and the treatment groups were sacrificed on 20^{th} gestational day. The fetal kidneys were harvested for both histo-morphological and stereological analysis. The quantitative data was collected using structured checklists, stored in excel spreadsheets windows 10, version 2016, then was exported for analysis into SPSS programme for windows version 25 for analysis (Chicago Illinois). The histo-photomicrographs were taken using a swift 3.0 microscope digital camera then uploaded to swift 3.0 software for labelling. Data analysis was done using ANOVA and MANOVA. Results were expressed as mean $\pm$SD, and all results whose $P<.05$ were considered significant. The study findings were as follows: in the treatment groups, there was a statistical significant reduction in the fetal growth parameters. Additionally, both omeprazole and pantoprazole affected the development of the fetal kidneys in a dose and time dependent manner particularly at TM_1 and TM_2, with omeprazole having more detrimental effects that included the altered renal histo-cytoarchitecture compared to pantoprazole. Further, both drugs led to a statistical reduction ($P<.05$) in the kidney volumes more so in the medium and high dose groups. Conclusion was drawn that both omeprazole and pantoprazole affected the development of the fetal kidneys in a dose dependent manner. It is recommended that medium and high dosages of the two drugs should be used with caution during

pregnancy particularly in TM_1 and TM_2. Further studies with non-primates closer to human are recommended to help collaborate these findings to humans.

CHAPTER ONE

INTRODUCTION

1.1 Introduction of the Chapter

This chapter starts by giving a brief introduction on the common uses of pantoprazole and omeprazole, plus a brief overview of gastroesophageal reflux disease (GERD), then gives a brief description of their teratogenicity mechanisms on the fetal viscera, this is followed by the problem statement, justification of the study, the study significance, research questions, study objectives, the hypothesis of the study, the aim of the study, assumptions of the study, the limitation, the delimitations of the study and conceptual framework.

1.2 Background Information

Omeprazole and pantoprazole are first generation proton pump inhibitors (PPIs) that are among the most commonly used medicines in the management of gastroesophageal reflux disease (GERD) in pregnancy (Law *et al.,* 2010; Nava-Ocampo *et al.*, 2006; Tytgat, 2001). One of the key reasons why pantoprazole and omeprazole are greatly used and at times abused during pregnancy is that, the gastroesophageal reflux disease (GERD) is one of the most common occurring and the most disturbing gastrointestinal disorder during pregnancy (Ali *et al.,* 2022; Lalkin *et al.,* 1998). However, the usage of these two medicines in pregnancy is riddled with controversy as some studies report that their safety use in pregnancy should be restricted while others reporting that they could be safe (Li *et al.*, 2020). This controversy is due to unclear findings on their safety profile on the developing fetal organ systems and in particular the fetal kidneys (Aykan & Ergun, 2018). Their mode of teratogenicity is that they cross the maternal placental blood barrier and block specific transporters that have protective role to the fetus against toxicity, produce phthalate coatings, and induce deficiencies in fetal morphogenesis, (Choi et al., 2023; Karttunen et al., 2017). The existing literature on the teratogenic effects of PPIs' have indicated that their use during pregnancy can lead to subclinical fetal

acute kidney injuries (AKI) that are not obviously diagnosed clinically and therefore can lead to chronic indolent renal damage after prolonged use (Xie *et al.*, 2017).

Furthermore, some other teratogenic studies have indicated that use of PPIs during pregnancy poses a high risk of congenital malformations to the developing fetal kidneys that is associated with inducing renal corpuscle injuries hence increasing the risk of fetal and adult renal diseases with increased risks of fetal mortalities in future (Aykan & Ergun, 2018). Though studies have pointed into possible teratogenic injurious effects of PPIs usage during pregnancy to the developing fetal kidneys (Ali *et al.*, 2022b), there is paucity of data on the the comparative histomorphological and histostereological teratogenic effects upon prenatal exposure of pantoprazole and omeprazole on the developing fetal kidneys. What is also not clear is whether or not the injurious teratogenic effects are dose and time dependent hence the basis of this study.

Gastroesophageal reflux is classified as a disease that has bothersome symptoms and complications because of the frequent reflux of stomach contents into the esophagus (Dong Seok *et al.*, 2021). It mainly manifests with epigastric pains, heartburn or regurgitation and nausea (Zielinski et al., 2015). Additionally, Gastro esophageal reflux disease is the most commonly clinical condition presented in health care facilities by the expectant mothers during their antenatal clinic (ANC) visits as it is reported by about 45 to 85% of the mothers visiting ANC clinics daily, (Body & Christie, 2016; Thélin & Richter, 2020). It is further reported that in every second, a pregnant woman suffers from GERD related symptoms, Malfertheiner *et al.* (2012) and will take a PPI such as pantoprazole or the omeprazole at any given period in the course of their pregnancy (Peron *et al*., 2023).

Pantoprazole is a medicine that bears various trade names that includes; protonix, prilosec, nexium (Pharmacokinetics, 2004). It is a proton pump inhibitor (PPI), that irreversibly binds and decreases secretions of gastric acid (Sampathkumar *et al*., 2013). It inhibits the hydrogen pump H^{+}/K^{+} ATPase irreversibly preventing the last and rate-limiting step in secretion of acid by parietal cells in the stomach (Makunts et al., 2019). Federation of drug administration (FDA) classify pantoprazole under

category B drug, meaning that it can be prescribed in pregnancy cautiously (Gerson, 2012). Similarly, omeprazole is a proton pump inhibitor (PPI) that is used for the control of acid secretion by inhibiting the gastric H^+K^+ ATPase (acid pump), an enzyme that functions in the final step of the hydrochloric secretion by the gastric parietal cell (Paz *et al.*, 2020). FDA classify omeprazole under category C category, meaning that it may be associated with adverse effects to the fetus as observed in past animal studies (Thélin & Richter, 2020). Both omeprazole and pantoprazole are commonly prescribed for management of epigastric pains associated with esophageal reflux during pregnancy (Dağlı & Kalkan, 2017) and are sold as prescription drugs as well as the over-the-counter drugs (Sampathkumar *et al*., 2013).

1.3 Problem Statement

The burden of kidney disease worldwide exceeds 850M people, and it is expected to be the 5th leading cause of years of life lost by the year 2040, (Jager et al., 2019), this constitutes a major public health challenge, (Brück et al., 2015). Additionally, congenital kidney anomalies account for approximately 30% of all anomalies identified in the prenatal period (Ckd & Graded, 2013; Seikaly et al., 2003). Out of these, ~50% leads to chronic kidney disease that require replacement therapy in children and young adults (Ckd & Graded, 2013). The proton pump inhibitors are reportedly the most commonly used and at times abused in high doses during pregnancy in the management of gastroesophageal refluxes as this condition is very commonly experienced by expectant mothers. (Good *et al.*, 2020; Gerson, 2012). Although omeprazole and pantoprazole are highly being used in pregnancy, their histomorphological and histostereological teratogenic effects on the morphogenesis of the fetal kidneys remains unclear. Furthermore, whether or not their teratogenic effects on the developing fetal kidneys are dose and time-dependent remains equivocal. The uncertain teratogenic risks that follows heavy usage of these two medicine by expectant mothers is happening in the wake of rising cases of juvenile and adult renal failures of unknown causes worldwide. Various morphological and functional effects can result if the developing kidneys are exposed to drugs like PPIs particularly during the critical period of early kidney development (Frazier, 2017). Whilst, the degree of safety for both pantoprazole and omeprazole is unknown, there

is associated risk of causing acute kidney injuries that may progress to chronic kidney injuries with resultant kidney failure (Freedberg *et al.*, 2019). On the other hand, pregnant women are being faced with challenges of gastric reflux and other digestive disorders prompting the use of these proton pump inhibitors (Gerson, 2011). Nonetheless, their relative teratogenic risks on the developing kidneys remains controversial with some studies showing either to be teratogenic and others showing they are not (Choi *et al*., 2023; van der Pol *et al*., 2011) and hence the need to establish relative histostereological and histomorphological teratogenic effects of these drugs on developing fetal kidneys, when used in different times and in varied doses during pregnancy.

1.4 Justification of the Study

Currently, there are increasing cases of acute and chronic kidney disorders of unknown causes across all age groups. At the same time expectant mothers are continuing to largely use and at times abuse proton pump inhibitors (omeprazole and pantoprazole) in management of gastroesophageal reflux, dyspepsia, among other conditions posing a great teratogenic risk to the developing fetal viscera including the fetal kidneys**.** The safety indexes of these two medicines on the developing fetal kidneys has remained vague as the past literature remain controversial on whether or not the two medicines are teratogenic and among the two the medicines which is safer (Diav-Citrin *et al*., 2005). The lack of comparative histo-quantitative teratogenic data to guide on the use of these two medicines will continue to pose a teratogenic risk to the developing fetal kidneys that predisposes them in the risk of progressing to chronic kidney failure during either childhood or in adulthood. In addition, scarcity of comparative teratogenic data that detail the most vulnerable teratogenic periods as well as the most critical doses of pantoprazole and omeprazole teratogenicity may deny mothers the benefits that would accrue use of either, in management of conditions like esophageal reflux, peptic ulcer disease which are common in pregnancy.

1.5 Study Significance

The histomorphological and histostereological findings of this study will be a useful guide on to which of these two drugs is safer to use during pregnancy when need be, and at what dosage. In addition, proper use of these drugs in the management of GERD during pregnancy, considering the time of administration will help minimize the chances of renal injury to the developing fetal kidneys. Additionally, the findings of the study will contribute to the scientific data repository in research as well as be a useful baseline for future studies on the higher non-human primates which would then guide more on the use of these drugs to the policy makers and by the clinicians on pregnant mothers.

1.6 Broad Objective

To comparatively evaluate the histomorphological and the histostereological teratogenic effects of *in-utero* exposure to varied doses of omeprazole and pantoprazole on the developing fetal kidneys when exposed at different gestational periods in albino rats (*Rattus norvegicus*).

1.6.1 Research Questions

1. What are the comparative effects of prenatal exposure to varied doses of omeprazole and pantoprazole on the maternal and fetal pregnancy outcomes in Albino rats?
2. What are the comparative histomorphological teratogenic effects of prenatal exposure to varied doses of omeprazole and pantoprazole when administered at different gestation periods on the developing fetal kidneys in Albino rats?
3. What are the comparative histostereological teratogenic effects of prenatal exposure to varied doses of omeprazole and pantoprazole on the developing fetal kidneys in the albino rats when given at different gestation period?
4. Are the comparative teratogenic effects of omeprazole and pantoprazole on the developing fetal kidneys of Albino rats dose and time dependent?

1.6.2 Specific Objectives

1. To evaluate the fetal and maternal pregnancy outcomes following prenatal exposure to varied doses of omeprazole and pantoprazole and at different gestation periods in Albino rats.
2. To evaluate the histomorphological teratogenic effects of prenatal exposure to varied doses of omeprazole and pantoprazole on the developing fetal kidneys at different gestation periods in Albino rats.
3. To evaluate histostereological teratogenic effects of prenatal exposure to varied doses of omeprazole and pantoprazole on the developing fetal kidneys at different gestation period in Albino rats.
4. To evaluate whether or not the observed effects of omeprazole and pantoprazole on the developing fetal kidneys of Albino rats are dose and time dependent.

1.7 Hypothesis of the Study

1.7.1 Null Hypothesis

There are no comparative significant differences in the histomorphological and the histo-stereological teratogenic effects of prenatal exposure to varied doses of pantoprazole and omeprazole when administered at different gestation periods on the development of the fetal kidneys in albino rats (*Rattus norvegicus*).

1.8 Study Assumptions

The current study assumes that the structure of the kidneys of the albino rats (*Rattus norvegicus)* and those of humans are similar microscopically and functionally. It also assumes that the development periods of the kidneys in humans and Albino rats is similar and hence any form of insult to the kidneys during the critical periods of their development may result to a similar injurious event in both rats and humans.

1.9 Study Limitations

1. Scarcity of peer reviewed journals on stereology

2. Unavailability of electron microscopy that would help in the detailed histocyto-architectural study of the kidney.

1.10 Study Delimitations

1. Study focused only on intrauterine life and not postnatal life of the kidney
2. Study only assessed the effects only on the kidney whereas the effects of the drugs could also have affected other organs.

1.11 Scope of the Study

This study entailed the prenatal exposure of two drugs to the developing fetal kidneys. The parameters that were studied included the following; the fetal and maternal pregnancy outcomes, the histomorphology alteration on the histological organization of the developing fetal kidneys; and histostereological teratogenic alterations on the developing renal structures of the fetal kidney in relation to doses applied as well as in relation to the time of exposures during the pregnancy**.**

1.12 Conceptual Framework

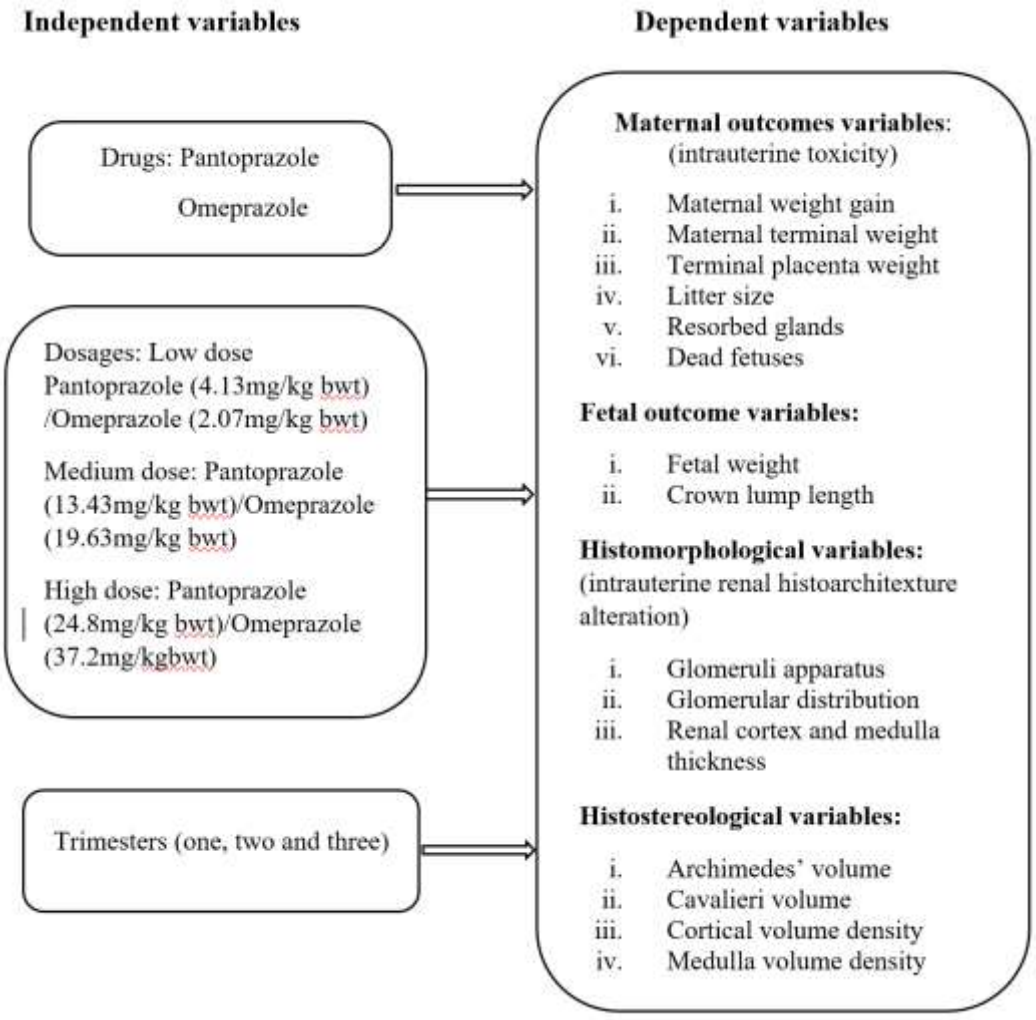


Figure 1.1: Conceptual Framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Chapter Introduction

This chapter begins by giving some brief pharmacological descriptions of pantoprazole and omeprazole in terms of their brand names, classes, chemical formula, mode of action, their solubility and mode of excretion. It then briefly describes their teratogenicity mechanisms on the fetal viscera, this is then followed by the descriptive comparative morphogenetic development of fetal kidneys between rats and humans, this is then followed by the general description of the maternal use of proton pump inhibitors (PPIs) vis a vis the maternal and fetal pregnancy outcomes, the known general histomorphological teratogenic effects of prenatal exposure to PPIs on the fetal viscera, the histo-quantitative teratogenic effects of PPIs in on the fetal viscera and finally whether the known general teratogenic effects of PPIs are dose and time dependent.

2.2. The Comparative General Description on the Pharmacology of Pantoprazole and Omeprazole

Pantoprazole that is sold under a number of trade names of Protonix®1, pantozol, pantoloc among others is a first generation proton pump inhibitor that is a substituted benzimidazole derivative (Shah *et al*., 2013). It has a molecular weight of 405.36 g/mol and is readily soluble in water (Kumar *et al.,* 2022; Shah *et al*., 2013) and is permeable to cell membrane. It works by decreasing gastric acid secretion by inhibiting the H+/K+-ATPase irreversibly, which is located within gastric parietal cells (Jewell, 2007). At the therapeutic dose of 40mg, it successfully lowers the secretion of the gastric acid (Fitton & Wiseman, 1996). It is available as an oral and intravenous formulation. It's also found in combination with drugs such as metronidazole, clarithromycin, or amoxicillin, used in the elimination of Helicobacter pylori (Cheer *et al.,* 2003). It is rapidly and completely absorbed after oral administration and almost exclusively metabolized via the cytochrome P450 (CYP) system in the liver (Smith *et al.,* 2021). Its main metabolic pathway is

demethylation by CYP2C19, with subsequent sulfation (Alaa *et al.,* 2019). Other metabolic pathways include oxidation by CYP3A4 (Ishizaki & Horai, 1999).

When administered orally, its bioavailability is approximately 77-79% and its absorption maybe affected by food (Ochoa *et al.,* 2020). Its absorption occurs in the small intestines, leading to maximum serum concentration of 2 to 3 hours post ingestion (Mathews *et al.,* 2010). The plasma half-life (t½) of pantoprazole is fairly short, about 1 hour. Its metabolism is independent of the route of administration and its metabolites are eliminated mainly through the kidneys, with a small percentage excreted via the faeces. It has a fairly lengthy duration of action on inhibition of acid due to its irreversibility and specific proton pump binding (Van Rensburg & Cheer, 2012).

Similarly, Omeprazole that is sold under the trade names losec, prosilec among others is a drug that is a substituted benzimidazole belonging to a class of proton pump inhibitors (Andersson *et al.*, 1991). It is a weak base, lipophilic, and therefore to a greater degree accumulates in an environment that is acidic like the parietal cell's secretory membrane (Lee & Kim, 2021). It has a molecular weight of 345g/mol. Omeprazole, being a first-generation PPI, is among the most prescribed drug in the management of GERD (Zhou *et al.,* 2022). It works by inhibiting the H^+/K^+-ATPase pumps (Soares *et al.*, 2021). It specifically inhibits H^+, K ($^+$)-ATPase, an enzyme that plays a key role in the last stage of acid secretion. Omeprazole inhibits acid secretion following stimulation of parietal cells (Robinson & Horn, 2003). It is metabolized in the liver and the excretion is through the urine (Cederberg *et al*., 1989). Its minimum dose in adults is 20mg, while its maximum dose 360mg. When administered at 20mg daily or more, it is able resolve the gastric acidity in many patients (Park *et al*., 2017; Howden, 1991).

2.3. The Comparative Morphogenetic Process of the Developing Fetal Kidneys between the Rats and Humans

The morphogenetic process of the developing fetal kidneys between rats and humans depict similar developmental milestones that make the rat models suitable in mimicking the developmental process of what would happen in humans. The human

kidneys develop from the fourth week of gestation through three stages; the pronephros, mesonephros, and metanephros. Pronephros, a rudimentary, non-functional kidney is the first stage of kidney development from the third week of development and disappears by the fifth week (Reidy & Rosenblum, 2009). Mesonephros forms at four weeks then essentially disappears. The metanephros are the ones that develops into the final functional human kidney (Rosenblum *et al.,* 2017). The mesonephric duct gives rise to ureteric bud which is an outgrowth that invades the mesenchyme of the metanephric during the fifth week of gestation, and then, goes through a series of successive branching and remodeling forming the radial structure of the kidney (Hartman *et al*., 2007). By the 34th week of gestation in humans, the renal collecting system is complete. This dictates final nephron complement.

Subsequently, the fetal kidneys in humans ascend from their position of initial development in the pelvis around the 6th to 9th week. They can be well seen via ultra sound done at the 12 to 13th week, with distinct renal architecture seen by the 20th week in which they show an external appearance that is lobulated. Urine is formed from around the 5 to 8th week; though, it is largely an unmodified plasma filtrate as tubular function starts around the 14th week (Nguyen *et al.,* 2010). Disrupted signaling that may alter patterning of the nephric duct, ureteric induction, or renal branching morphogenesis is likely to lead to diverse malformations (Blake & Rosenblum, 2014).

On the other hand the fetal kidney morphogenetic process in rats involves the formation of the fetal kidneys that are in a well-regulated balance amid proliferation, differentiation, apoptosis, and morphogenesis process (Frazier, 2017). The fetal kidneys development in rats is similar to those of human developmental stages with only some differences in the time points in which different renal structures varies in rats and humans. The kidney develops in three stages that are pronephros, mesonephros and metanephros or the adult kidney (Rosenblum *et al.,* 2017). Pronephros which is the first stage in development of the kidney and appears around gestational day 11 in the rats and day 22 in humans. Mesonephrons forms though degenerates later. This is followed by the formation of metanephric kidney from the

extension and branching of the ureteric bud into the metanephric mesenchyme commencing the formation of nephrons (Seely, 2017).

Morphologically, development of the kidneys in humans occurs entirely in utero. Formation of nephrons and organogenesis occurs from 6–36 week of gestational period and thereafter, nephrogenesis is complete (Solhaug *et al.*, 2004). Although in humans fetus; nephrogenesis begins and is accomplished afore birth, in the rat it continues after birth and is completed around 11–15th day postnatal (Zoetis & Hurtt, 2003). Full functional maturation of the kidneys differs among species and ranges from nearly one month in rodents to almost two years in humans (Bueters *et al.,* 2020)**.**

2.4 The Known Teratogenic Effects of Prenatal Exposure to PPIs on the Developing Fetus and the Fetal Kidneys.

Existing literature has shown that prenatal exposure to PPIs is associated with a myriad of congenital structural defects to the developing fetal viscera that includes the fetal kidneys. In a study done by Mubeen et al. (2016), noted that, the structural cells in the fetal kidneys were seen to bear significant injurious effects when exposed to a groups of PPIs due to Prenatal insults and premature birth that was also associated with mal-development of the fetal kidneys. Literature is pointing to the fact that, use of proton pump inhibitors is associated with a high likelihood of acute kidney injury, probably due to acute interstitial nephritis. In a population-based study of people who had used different PPIs, the rate of acute kidney injury and acute interstitial nephritis were 2.5 to 3-fold higher in PPI users compared with proton pump inhibitors' naïve patients (Schoenfeld & Grady, 2016). Omeprazole belongs to class C medicines that should be applied with caution during pregnancy according to FDA and embryo-toxic and fetotoxic effects have been reported in the animal studies. Cases reported in humans suggest similar concerns. In an observational study, Broussard *et al.* (1998), reported that two consecutive pregnancies had been terminated due to an anencephalic fetus and severe talipes of the fetus in a woman who had taken omeprazole 20 mg/day for severe reflux esophagitis prior to conception (Broussard & Richter, 1998). Experimental evidence in rats concerning

other organs suggests that administration of PPIs limits the regenerative capacity of the liver after partial hepatectomy. Furthermore, it is unclear whether exposure to PPI also limits the regenerative capacity of renal tubular cells following injury*(Xie et al.,* 2017).

Another study found out of the 113 thirteen pregnant women exposed to omeprazole during the duration of pregnancy, the rates of major malformations (4%) in the those who had taken omeprazole did not differ significantly from controls who were exposed to non-teratogens (2%) and disease-paired control, this contrasts the previous study stated as above (Lalkin *et al.,* 1998). According to federation of drug administration (FDA), most PPIs are categorized as class B drugs apart from omeprazole because of its fetal toxicity which is classified as a class C drug. Some birth defects such as anencephaly and hydranencephaly have reported to FDA in pregnant women who had taken omeprazole for the pregnancy period. However, other researchers reported taking 20-60 mg omeprazole/day had no risk of fetal congenital anomalies even if taken from the first trimester (Alaa *et al.,* 2019).

2.5 The Observed General Histomorphological Teratogenic Effects of Prenatal Exposure to PPIs' on the Developing Fetal Kidneys

The existing literature has shown that the histological organization of the developing kidneys are sensitive to chemical teratogens like PPIs that usually do cause both morphological and functional disturbances to the early developing fetal kidney morphogenesis up to the postnatal phases of growth and differentiation (Frazier, 2017). Kidneys play a very important role in the plasma filtration making them to be more prone to renal toxicity of the drugs. In addition, the H/K ATPase pump is also found in the distal uriniferous tubules of kidney and these pumps are sensitive to omeprazole (Mubeen *et al.,* 2016). In studies done with animals such as rabbits, death of embryo, fetal resorption and pregnancy disruption have been reported with doses of some PPIs when administered at higher than the usual human dose. Similarly, fetal toxicity in rats was noted following administration of the drug high doses than the usual human dose (Broussard & Richter, 1998).

In another study about effects of PPIs that included pantoprazole, to pregnant rats, the results demonstrated growth retardation of the fetus. There were also hematomas noted in the fetuses and high fetal resorption, skeletal anomalies on fetus, decreased ossification in many bones and costal separation among other malformation. Similarly, a decrease in the level glutathione, an antioxidant was also noted (Alaa *et al.*, 2019).

2.6 The Known General Histo-Stereological Teratogenic Effects of *In-Utero* Exposure to PPIs of the Developing Fetal Kidneys.

Previous studies have reported that prenatal exposure to a wide array of PPIs have been associated with causing alterations in the morphogenetic histological organization of the various renal structures of the developing fetal kidneys (Xie *et al.*, 2017). The reported histological alterations spans from the changes in the glomerular distribution, the alterations in the thickness of the renal cortex and medulla. Such histological changes in the developing fetal kidneys was shown to occur when different doses were administered at different times, with frequent dosing being associated with the risk of chronic kidney disease which was observed in 50% higher in PPI users, compared with the naïve patients and by a dose response with higher risk among patients who took the drug twice daily compared with once daily dosing of PPI (Schoenfeld & Grady, 2016). In another study, it was noted that use of Proton pump inhibitors (PPI) was led to an increased risk of acute kidney injury, incident chronic kidney disease (CKD), and progression to end-stage renal disease (Xie *et al.*, 2017). Conversely, another study, found there was no associated increase in the congenital malformations in 1,186 infants and abortuses that had been exposed to PPIs at the first trimester of pregnancy. Moreover, no increased risk of perinatal mortality, premature delivery, low birth weight, or low Apgar scores observed following exposure to PPIs during the third trimester of pregnancy (Matok *et al*., 2012).

2.7 The Reported Teratogenic Effects of Prenatal Exposure to PPIs in Relation to the Doses and the Time of Exposure on the Developing Fetus and kidneys.

Studies that have been done previously on the teratogenicity of PPIs have reported mixed teratogenic effects of PPIs in relation to the time and the dosages of exposure. Some studies showing that the PPIs teratogenicity have a dose and time dependency relationship while others showing only relation to dose or relation to time only. What is clear across all the teratogenic reports from different studies is that, the differences' in functional and morphologic timing during the different stages of fetal kidney development across different species can aid in describing the precise appearances of different intrauterine and postnatal teratogens and their nephrotoxins on the developing fetal kidneys (Frazier, 2017). A cohort study showed that, use of omeprazole in the first trimester was associated with 52 (2.9%) major birth defects among 1800 live births (Pasternak & Hviid, 2011). Further, in patients who had an exposure to PPIs among the live births; there were reported major birth defects in infants born to mothers who had been exposed to PPIs four weeks prior conception till the end of the trimester one as compared those mothers were not exposed (Pasternak & Hviid, 2011). Similarly, 21 (3.8%) birth defects were reported among 549 live births in a study analysis that only focused on exposure pantoprazole in the first trimester (Thélin & Richter, 2020).

Considering the possible mechanisms associated with the kidney damage caused by the PPI, Kamal *et al.,* (2018) identified that the use of some PPI such as omeprazole may be associated with a long term effects occurring from recurrent acute processes that may involve deposition of the drug and its metabolites in renal tissue as the blood goes though renal system for plasma filtration, which may result in renal interstitial fibrosis, leading to chronic lesion and onset of CKD.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Chapter Introduction

This chapter starts by first describing the study setting. This is then followed by the study design, the description of the study subjects, the sample size determination, the grouping of the animals, inclusion and exclusion criteria, the feeding of Albino rats, breeding and confirmation of pregnancy, determination, calculation and administration of pantoprazole and omeprazole, duration of pantoprazole and omeprazole dose exposures, the humane sacrificing of pregnant Albino rats, harvesting of fetuses, harvesting of the fetal kidneys, histomorphological and stereological procedures, data analysis, ethical considerations and approvals.

3.2 Study Setting

This study was done in two study settings; The first study setting was in the Department of Biological sciences in Chiromo campus of the University of Nairobi. This is where all the procedures that involved animal handling and animal experimentations were carried out including; the breeding of rats, the mating, feeding, weighing and administration of drugs till the harvesting of fetuses and the harvesting of fetal kidneys for histomorphological and histo-stereological analysis, the second study setting was the in JKUAT, School of Medicine and in particular the histology lab in the department of human anatomy, where the processing of the kidney tissues for light microscopy and for histo-stereological analysis was done. In these laboratories, there was well trained laboratory staff who assisted the researcher in animal handling and well maintained humid conditions and the 12hrs light dark cycles. Additionally, there was availability of well-maintained equipment such as high precision weigh scales.

3.3 Study Design

In carrying out this study, a post test only with control experimental study design was adopted. This study design was considered suitable for this type of a study in that all

the histo-morphological and histo-stereological teratogenic effects on the developing fetal kidneys were analyzed after the prenatal exposures to pantoprazole and omeprazole and after harvesting the fetal kidneys.

3.4 Study Subjects

A total of 30 female Albino rats aged 7 to 8weeks old, weighing between 190 to 230 grams were sourced from the Lower Kabete - veterinary medicine, University of Nairobi, and these were used as a study model because of the following scientific facts; (i) The albino rats are approximately 90% identical to humans at their genetic makeup which contributes to a higher success rates in animal research (Shanks et al., 2009). (ii) they have a large litter size ranging between 3-14 fetuses, (iii) they have fewer chances of spontaneously occurring congenital defects, (iv) they have a relatively short gestational span, making it easier to get study subjects or a pure breed colony (v) low cost of maintaining the animals, (vi) they are plentiful, (vii) considerable amount of the reproductive data on the rat was already available, (viii) they are relatively small and easy to care for and handle during an experiment (ix) they are relatively resilient in terms of withstanding a wide range of study medicines, (Bailey *et al.*, 2014; Pritchett & Corning, 2016).

3.4.1 Brief Description of Albino Rats.

The Albino rats (*white rat* or *Daikoku* rat) originated from the breeding of the hooded rats, believed to have its origin in Japan, and have been in use as the "laboratory rat" since the mid-19th century (Takashi, Kuramoto (Institute of Laboratory Animals, Graduate School of Medicine, 2012). Due to their gentle nature, these Albino rats were the first to be domesticated for use in scientific based research (Kuramoto et al., 2012), and ever since then they have become the laboratory animal for research. In scientific research, these Albino rats are used as models to humans. These Albino rats usually have all white hairs with red eyes, and a long tail. They mature sexually at around the 6th week, have estrous cycle in which is time that the rat is sexually active (Benjamin, 2019). Additionally, they possess unique features that make them an ideal for scientific research.

3.5 Sample Size Determination

The resource equation for one-way Analysis of Variances (ANOVA) method was used in determining sample size because the standard deviation from previous studies and the effect on size was not available (Arifin & Zahiruddin, 2017). In this method, the value 'E' was measured which was the degree of freedom of analysis of variance (ANOVA) based on a decided sample size. This value ('E') lies between 10 and 20 rats. A value less than 10 necessitated adding more animals which would have increased the chance of having significant results. A value more than 20 has been shown to increase the cost of the study without increasing the significance of the results (Charan & Biswas, 2013).

Formula; $(n = DF/k + 1)$, $N=n \times k$

N=total number of animals

k=total number of groups

n= number of rats per group

n=20/10+1=3

Total number of rats= 10 groups x 3rats per group =30 rat dams, these were picked by use of convenient sampling method.

Since, every adult female rat was assumed to have a minimum average of three (3) fetuses per pregnancy; fetuses from each of the 30 rats were ordered according to the body weight, then by use of systematic uniform random sampling, three fetuses were, 3 fetuses were selected to make a total of 90 fetuses (30x3). Additionally, 15 sexually mature males were picked from the pure colony of the same species of Albino rats and were used for mating purposes.

3.6 Breeding of the Rats

Breeding of the rats for use in the study was done at the animal house located at the university of Nairobi. This was done by breeding the rats until the 4^{th} series of breed

was achieved. A sample size of 30 rats was then selected from this serially bred colony, for use as a pure colony for this study and additionally 15 male rats that were used for mating.

3.7 Mating of the Rats

Before mating, the male albino rats from a pure colony of the 4th series and sexually mature (7-8 weeks old), were put in a polycarbonate plastic cage separated by wire mesh from the female albino to acclimatize before mating. Acclimatization was allowed for a period of five days. Afterwards, one male was introduced into a standard cage with two female rats that were in their estrous cycle and were allowed 24 hours of light and dark cycle after which, the males were then removed and returned to their separate cages.

3.8 Pregnancy Determination

Pregnancy was determined at two levels as follows;

Level 1: Confirmation of Mating

Vaginal smears were taken from the mated female rats where presence of spermatozoa on the smear were observed under the microscope, this confirmed that coitus had taken place.

Level 2: Confirmation of Pregnancy

In this level, the vaginal smears were be taken from the 30 mated females the next morning and pregnancy was determined by doing a vaginal swab where presence of polyhedral epithelial cells on the swab was used to determine estrous changes, which was denoted as the first day of gestation (GD_1) (Heyne *et al.*, 2015).

3.8.1 Materials Used in Determination of Pregnancy

i) Cotton tipped swab
ii) 0.85%phosphate buffered saline
iii) Microscope slides

iv) Ethanol (95%)
v) Absolute alcohol
vi) 10mls blunt tipped disposable pipettes
vii) Giemsa stain

The Procedure that Was Followed in the Determination of Pregnancy

1) The rats were restrained with a gauze holder against the body.
2) 1ml of saline was introduced into the vaginal cavity using a blunt tipped disposable pipette
3) Cotton tipped swab moistened with phosphate buffered saline was gently inserted into the vaginal cavity
4) The swab was slightly rolled before withdrawing
5) The moist swab was withdrawn and rolled onto a clean glass microscope
6) The specimen was then fixed using 95% ethanol spray
7) Then the slides were air dried and others by dipping in 100% alcohol
8) The slides then were stained with giemsa stain
9) The slides were observed under the Light microscope (optika).

3.8.2 Observations to Confirm Fertilization

Presence of large, polyhedral epithelial cells, many neutrophils on the smear and scattered epithelial cells served as an indicator that fertilization had taken place and this was counted as the first day of pregnancy (gestation day one). Those that had not conceived, were allowed for another 24hours with the males after which the test was repeated again to confirm their pregnancy.

3.9 Selection Criteria

3.9.1 Inclusion Criteria

a. Rats that conceived in the first day after being introduced to a male overnight
b. All the pregnant rats that never shown any sign of sickness

c. All fetuses that were alive at the point of sacrificing the animals and opening the uterus.

3.9.2 Exclusion Criteria

a. All rats that didn't have a positive pregnancy test following the introduction of a male
b. Any dead fetuses during the time of opening the uterine horns

3.10 Grouping of the Rats

The 30 rats used in the study were randomly assigned to either 3 rats as the control and 27 rats in the experimental category. To determine whether the effects of omeprazole and pantoprazole were dose dependent, the 27 rats in each experimental category were divided into three broad study subgroups of 9 rats each based doses applied as follows: 9 rats for the low dose of omeprazole/ pantoprazole group; 9 rats for the medium dose of omeprazole/pantoprazole group; and 9 dams for the high dose of omeprazole/pantoprazole group. To determine whether the effects of omeprazole/ pantoprazole are time dependent, the 9 rats in each of the three study subgroups of the low, medium and high doses of omeprazole/pantoprazole were further subdivided into three subgroups of 3 rats each based on the trimester of exposure as follows; 3 rats for trimester one (TM_1), 3 rats for trimester two (TM_2) and 3 rats for trimester three (TM_3) **(**Figure 3.1).

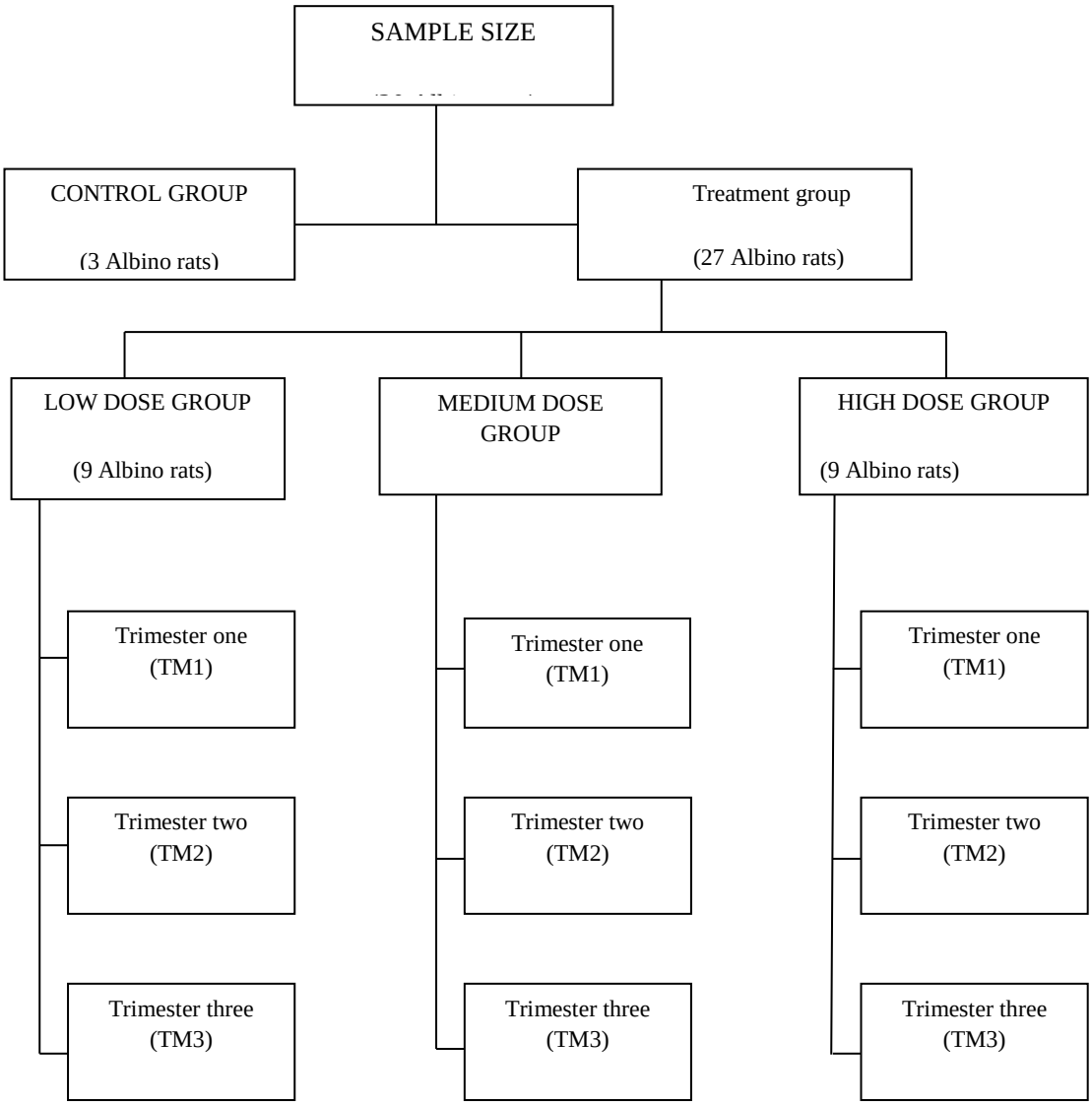


Figure 3.1: An Illustration of How the 30 Albino Rats Were Grouped into Both the Control and the Treatment Groups

3.11 Feeding of the Rats

The female Albino rats were kept in spacious polycarbonate plastic cages which measured 17 × 36 × 23 cm, that had bar lids to hold the feeds and water bottles. They received water *ad libitum,* and the rodent pellets obtained from UNGA meals limited which comprised of 68% starch, 4% cellulose, 5% lipid (corn oil) and 20% protein

per a (100 g). These rodent pellets and clean water were put every morning at 0800hrs, in the spacious polycarbonate plastic cages for daily feeding as outlined by (Allen *et al.,* 2016).

3.12 Handling and the Care of the Rats

Prior to handling the rats, the researcher and the assistant researcher underwent training regarding animal handling which was done at JKUAT SAFARI animal house. All procedures entailing the handling of the rats were performed according to the guidelines for care of laboratory animals by the National Institute of Animal Research (Choong, 2003) and National Research Council, report of 2011. There was daily cleaning of the room and the polycarbonate plastic cages, and checking for any breakage of the cage in which case it was replaced, as well as changing the bedding so that the rats were comfortable. Water bottles were cleaned daily and thereafter refilled with clean and safe water for the rats. The rats were weighed daily between 0700 hrs and 0745 hrs using a high precision weigh scale and weight was recorded down in the structured checklists. Their behavior was also observed every day so as to identify any abnormal behavior that would have suggested the possibility of an illness.

In addition, a consistent environmental conditions of the temperature, humidity control and 12-hour light/dark cycle was maintained.

3.13 Occupation and Safety Measures

The occupation and safety measures were observed so as to ensure the safety of the researcher and also for the study rats. For the researcher, a safety precaution measure chart was mounted on the wall in case of any accidental events. It was ensured that the researcher washed her hands and donned in protective gears every time she was handling the rats. These included the clean lab coat, gloves, nose masks and closed shoes. There was disinfectant always put at the doorway, in which the researcher would always step on before and after entering into the animal house so as to prevent chances of spreading the microorganisms. Additionally, the researcher exercised great care and gentleness when handling the rats during the weighing and drug

administration so as to prevent them from becoming aggressive, hence minimized chances of bites and scratches from them. The polycarbonate plastic cages were maintained in good conditions such that there were no broken or sharp edges and no study rat was allowed to move from one cage to another in the entire study time, so as to ensure and reduce any chance of infection.

3.14 Drug Administration

The drugs of this study were administered every morning at 0900 hrs to the experimental rats using a gavage gauge 16. Additionally, the control group received a water at the same milliliter as was used to reconstitute the drugs.

3.14.1 The Low Dose Pantoprazole /Omeprazole Groups

The rats in the two treatment groups of low dose received a constant daily dose of omeprazole 2.06 mg/kg and pantoprazole 4.13 mg/kg respectively administered by use of a gastric gavage gauge 16, at 0900hrs. The 3 rats in trimester one (TM_1) were receiving omeprazole or pantoprazole treatment daily from day one (GD_1) to gestation day twenty (GD_{20}); those in trimester two (TM_2) received the treatment daily starting from gestational day eight (GD_8) all trough to gestation day twenty (GD_{20}), while those in trimester three (TM_3) received daily pantoprazole/omeprazole treatment daily from gestational day fifteen(GD_{15}) all through to- gestational day twenty (GD_{20}).

3.14.2 Medium Dose Omeprazole/Pantoprazole Groups

The experimental rats in this dosage group constant daily dose of omeprazole 18mg/kg/ pantoprazole 11 mg/kg respectively, administered once in a day through the use gastric gavage gauge 16 at 0900 hrs. The 3 rats in trimester one (TM_1) received omeprazole or pantoprazole treatment daily from day one (GD_1) to gestation day twenty (GD_{20}); those in trimester two (TM_2) were receiving the treatment starting daily from gestational day eight (GD_8) all through to gestation day twenty (GD_{20}), while those in trimester three (TM_3) received treatment daily from gestational day fifteen (GD_{15}) all through to the gestational day twenty (GD_{20}).

3.14.3 High Dose Omeprazole/ Pantoprazole Groups

The experimental rats in this dosage group constantly received a daily dose of omeprazole 37 mg/kg/ pantoprazole 25 mg/kg respectively administered once a day through gastric gavage gauge 16 at 0900 hrs. The 3 rats in trimester one (TM_1) were receiving omeprazole or pantoprazole treatment daily from day one (GD_1) to gestation day twenty (GD_{20}); those in trimester two (TM_2) received the treatment starting daily from gestational day eight (GD_8) all trough to gestation day twenty (GD_{20}), while those in trimester three (TM_3) received daily omeprazole treatment daily from gestational day fourteen (GD_{15}) all through to- gestational day twenty (GD_{20}).

3.15 Acquisition, Calculation of Doses and Administration of the Drugs to The Experimental Rats.

The adult Omeprazole dosages in human ranges between 20 mg-360 mg per day while pantoprazole ranges between 40-240 mg in divided dosages. Both medicines were obtained from a registered chemist in Nairobi, considering their batch numbers and were reconstituted using distilled water.

3.15.1 The Calculation the Drug Dosages

A simple guide for conversion of animal dosages from human dosages was applied (Nair *et al.*, 2018), which states that;

- Animal equivalent dose (AED), (mg / kg) = Human dose (mg / kg) × K_m ratio
- The Km factor for rats is 6.2, then we multiply human equivalent dose in mg/kg.

3.15.2 Calculation of Omeprazole and Pantoprazole Doses

The maximum omeprazole dose in humans is 360 mg, medium dose is 190 mg and minimum dose is 20 mg, while the average weight of an adult human is approximately 60kg, (Walpole *et al.*, 2012).

On the other hand, the lowest dose for pantoprazole in humans is 40 mg, medium dose is 110 mg while the highest dose is 240 mg.

1. Calculation of Omeprazole Dosages

a) Determination of High Dose Omeprazole Group

Highest dose omeprazole – 360 mg

Average weight of a man – 60kg

360 mg = 60kg

X=1kg

X=1x360/60 = 6 mg/kg

Animal equivalent dose (AED) = Human equivalent dose (HED) x Km factor

Therefore, 6 mg/kg x 6.2 =37.2 mg/kg BW

1kg =1000 gm; thus 1000 g= 37.2 mg, therefore, 1 g = 37.2/1000= 0.00372 mg

- So, the weight of the rats in grams was multiplied by 0.00372 to get the highest dose for omeprazole.

b) Determination of Medium Dose Omeprazole Group

Medium dose omeprazole – 190 mg

Average weight of a man- 60kg

190 mg = 60kg

X=1kg

X=1x 190/60 = 3.1667 mg/kg

AED = HED X Km factor

Therefore, 3.1667 mg/kg x 6.2 =~19.6333 mg/kg BW

c) Determination of Low Dose Omeprazole Group

Lowest dose Omeprazole – 20 mg

Average weight of a man - 60kg

20 mg = 60kg

X = 1kg

X=1x20/60 = 0.033 mg/kg

AED = HED X Km factor

Therefore, 0.033 mg/kg x 6.2 = ~ 2.07 mg/kg BW

2. Calculation of Pantoprazole Dosages

a) Determination of High Dose Pantoprazole Group

Highest dose Pantoprazole – 240 mg
Average weight of a man - 60kg
240 mg = 60kg
X=1kg
X=1x240/60 = 4 mg/kg
AED = HED X Km factor
Therefore, 4 mg/kg x 6.2 =~24.8 mg/kg BW

b) Determination of Medium Dose Pantoprazole Group

Medium dose pantoprazole – 130 mg

Average weight of a man - 60kg

130 mg = 60kg

X=1kg

X=1x 130/60 = 2.167 mg/kg mg/kg BW

AED = HED X Km factor

Therefore, 2.167 mg/kg x 6.2 =~ 13.4354 mg/kg BW

c) Determination of Low Dose Pantoprazole Group

Lowest dose pantoprazole – 40 mg

Average weight of a man - 60kg

40 mg = 60kg

X=1kg

X=40/60 = 0.667 mg/kg

AED = HED X Km factor

Therefore, 0.667 mg/kg x 6.2 = 4.1333 mg/kg BW

- Since the weight of rats to be used in the study range between 190-230 g, then the dosage needs to be converted into mg/kg to mg/g as follows;

3. Calculation of Specific Rat Dosages

If for example the weight of the rat is **200 g** and low omeprazole dose **2.067 mg/kg,** then calculation is done as follows;

(2.07 mg/kg/1000) = **0.002067 mg/g**

0.00207 mg/g x200 g = **0.414 mg**

If omeprazole tablet is **20 mg,** and reconstitution is done in **10 ml** of distilled water, **then**

20 mg=10 ml

0.4 mg=

$\frac{0.414\ mg \times 10\ ml}{100\ mg}$ = **0.0414 ml**

3.15.3 Administration of Omeprazole and Pantoprazole

Both omeprazole and pantoprazole was administered by the researcher on daily bases at 0900 hrs.

a) Materials Required for Administration of Omeprazole

i) Pregnant dams (30)
ii) Tabs omeprazole and pantoprazole
iii) Gavages' needle gauge 16
iv) 20 ml beaker for dilution
v) Syringes-2 ml and 5 ml
vi) Distilled water (500 mls)

vii) A table cloth

b). The Procedure for in Administering Various Doses of Omeprazole and Pantoprazole Using Gastric Gavage

1) Each respective rat according to the dosage level, was carefully held from the neck region using the left hand.
2) The rat was wrapped with the table cloth to avoid the animal from soiling the researcher clothing's
3) It was then rested against the body with the animal mouth facing the researcher
4) The gavage needle gauge16 was gently inserted into the mouth of the rat turning it gently to pass the esophageal constrictions
5) The treatment was then put in the stomach of the rat
6) The gavage needle then was gently removed
7) The rat was then carefully and gently returned to its cage.

3.16 Humane Sacrificing of the Pregnant Albino Rats

All pregnant rats were humanely sacrificed by inhalation of concentrated carbon dioxide between 0900 HRS and 1100 HRS at gestational day 20^{th} so as to avoid devouring dead fetuses or any congenitally deformed fetus.

(i) Materials Used for the Humane Sacrificing of Rats

a) The pregnant rat dam of gestation date 20^{th}
b) Concentrated carbon dioxide (CO_2)
c) Cotton wool
d) Bell jar
e) Physiological saline 0.85% concentration
f) Mounting board
g) Mounting pins
h) A pair of scissors
i) A pair of forceps (toothed)
j) Scalpel blade
k) Scalpel blade handle

l) Fixative- 10% formaldehyde
m) 2 drip sets
n) Normal saline
o) Hypodermic needle gauge 20
p) Clean gloves
q) High precision electronic weighing scale
r) Specimen collection bottles

(ii) Procedure for Humane Sacrificing of the Rats

a. Concentrated carbon dioxide was introduced into a bell jar
b. A tight fitting lid was then put into the bell jar
c. The pregnant rat was put into the bell jar and the lid was fitted back tightly
d. The rat was waited for 10-15 minutes to be euthanized and anaesthetized
e. The rat was then removed from the bell jar and mounted onto the board using mounting pins with dorsal side on the board
f. Using a pair of scissors and forceps the rat was cut through the ventral medial side from the xiphi-sternal joint to the symphysis pubis.
g. The perfusion needle was inserted to the left ventricle of the heart while connected to the perfusion set containing 400 mls of normal saline
h. The blood was cleared from the rat with physiological saline (200 mls of 0.85mol/litre) through the left ventricle of the heart (saline flew by force of gravity from one of the drip set)
i. After sufficiently clearing, the saline drip was removed (the needle then left in position of the heart and the fixative formaldehyde 10% was introduced.
j. The firmness of the tail was checked as a sign of effective fixation of the animal
k. The drip was disconnected and the perfusion needle removed from the heart

l. It was immersed it in a container with 10% formalin to continue with fixation for 24 hours

3.17 Harvesting of Fetuses

i. Twenty minutes after euthanizing and anesthetizing the rats with concentrated carbon dioxide, the abdominal wall of the mother was opened from the xiphi-sternal joint to the symphysis pubis along the linear alba and the full extent of both uterine horns exposed promptly.
ii. Before opening either of the placental horn, fetal positions within the horns as well as the number of live and dead fetuses indicated by their movement following a gentle prodding with a probe was determined and recorded as litter size.
iii. The number of the "resorped endometrial glands" was characterized by yellowish nodules found along the anti-mesomentrial margin of the uterine horns that marked any original implantation site was counted and recorded. Thus, the endometrial glands unoccupied by living or recently dead fetuses represented the number of prior resorptions.
iv. The uterine horns were excised along the anti-mesomentrial border to expose the fetuses, embryonic membranes and placentas using a pair of scissors.
v. They were gently removed in totality from the uterus, utilizing the blunt end of a pair of forceps.
vi. An incision along the dorsal surface of the membranes revealed the fetuses,
vii. Each fetus and its placenta was removed, weighed and the general fetal morphology examined and recorded immediately.
viii. General examination was done to check for any abnormalities
ix. The fetal length and crown-rump length, for each fetus was be taken and recorded
x. Then the fetuses were then sacrificed to harvest the kidneys

3.18 Harvesting the Fetal Kidneys

From the three fetuses obtained from the 30 rats, their kidneys were harvested for both histostereological and morphometric analysis according to the following procedure;

a) Fetuses were mounted onto the dissection board using mounting pins in supine position.
b) Using a pair of scissors and forceps the midline abdominal incision to open the abdomen.
c) Using a magnifying glass, the whole fetal kidneys were identified.
d) To avoid damaging the fetal kidneys, they were carefully held using the forceps.
e) Each kidney was excised as a whole together with the adrenal glands at the level of renal pelvis and there after separated from the gland
f) Each kidney was examined for general external features and obvious congenital malformations
g) Kidney weights were taken by use of a high precision weighing scale.
h) Kidney length, width and thickness were assessed using Vernier caliper and a ruler.
i) The kidneys were immersed in the formaldehyde, to proceed with processing either for light microscopy or stereology for 24 hours.

3.19 Processing for Light Microscopy

a. Materials Used for Tissue Processing

i. The specimens (the fetal kidneys)
ii. Glass slides and cover slips
iii. Hematoxylin and eosin
iv. Glass staining square jars
v. Paraffin wax
vi. Microtome knives
vii. Rotary microtome
viii. Heater and water bath container

ix. Specimen bottles
x. Slide holders
xi. Distilled water
xii. Formaldehyde 40%concentration
xiii. Xylene
xiv. Isopropyl alcohol
xv. Wood blocs
xvi. Glass ware for preparation of dilutions
xvii. Beakers
xviii. Egg albumin
xix. Dropper
xx. Cedar wood oil

b. The Procedure for Processing the Fetal Kidneys for Light Microscopy and Stereology

1) The kidneys were fixed in formaldehyde) for 24 hours
2) They were then dehydrated in an ascending concentration of alcohol (50%, 60%,70%, 80%, 90% - each for 15mnutes and then 100% and 100% (absolute) each for 30 and 45 minutes respectively.
3) The dehydrated kidneys were then cleared by use of xylene for
4) Once cleared, the fetal kidneys were then infiltrated with molten paraffin wax for at 56^0c 12 hours
5) The kidney tissues were then orientated and then embedded in paraffin wax on the wooden blocks and allowed to cool
6) Excess wax was trimmed-off till the entire length of the kidney tissue was exposed
7) 5µm thick longitudinal sections were cut using Leitz sledge rotary microtome
8) The cut sections of the kidney were then floated in water at $37^{\circ C}$ to spread the tissue
9) The sections were then mounted using egg albumin, applied as thin film with a micro-dropper.
10) The slides were dried in an oven at 37^0 C for 24 hours

11) Blinding was done by coding all the slides by the research assistant in absence of the researcher
12) They were stained using Hematoxylin and Eosin (H&E), based on the cellular structures that needed to be studied by the use of the light microscopy.

3.20 Stereological Analysis

3.20.1 Estimation of the Initial Total Kidney Volume Using Archimedes Principle

Immediately after harvesting the fetal kidneys from the control and experimental groups, the total initial kidney volumes were determined using the Archimedes' principle. In this method, the kidneys were inserted into graduated beakers containing normal saline, and the amount of fluid displaced was measured to represent each initial kidney volume (Mohazzab, 2017).

3.20.2 Determination of Total Fetal Kidney Volume (Cavalieri Point Counting Method) Using Stepanizer Stereological Tool

The terminal total kidney volume was estimated by use of Cavalieri point counting method by which the following steps were used as per (Cruz-Orive, 1999).

i) Preparation of each kidneys' Cavalieri sections (5µ) thick sections
ii) Selection of the spacing for the point probe
iii) The point probe was tossed randomly onto each section
iv) The points that hit the region of interest were counted using STEPanizer stereology tool
v) All sections were processed keeping a tally of counts per section
vi) The formula was applied to calculate the volume.

Twenty sections of 5µm thickness from each longitudinal kidney section were selected by systematic uniform random sampling, beginning from a random start (Elfil & Negida, 2019). Using the microscope's stage Verni, the entire kidney slices were viewed at magnification of X10 and digital images captured using swift 3.0

camera (SC2003) and saved in the joint photograph expert group (JPEG) file format at adequate resolution. The photos were then uploaded in the computer screen in the STEPanizer tool for point counting using stereological sampling rules ***(Figure 3.1).***

Where stereological estimation required the use of a guard area it was set and was changed in the course of the whole experiment to obtain consistent results. Volume of the fully sectioned kidney was the product of the sum of the cut areas (starting with the first to the last section (Journal, 1981). All the fields of both kidneys were selected and images projected on a computer screen. A transparent test system on the grid was then superimposed on the images projected, and points hitting these areas were counted.

The formula used was as follows;

$$\hat{V} = A_p m' \bar{t} \left(\sum_{i=1}^{n} P_i \right)$$

Figure 3.2: An Illustration of the Cavalieri Formula

Key

A_P: *is the Area associated with a point*
m': *Is the section evaluation interval*
t bar: *Is the mean section cut thickness*
p_i: *Are the points counted on the grid*

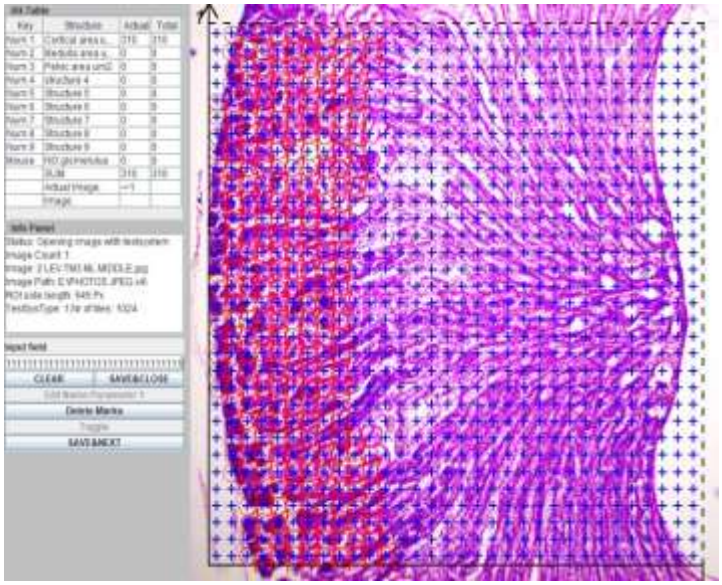


Figure 3.3. A Photomicrograph of the Point Counting Frame for the Kidney Done Using a Stepanizer Tool for Cavalieri Volume Determination.

3.20.3 Stereological Correction for Kidney Tissue Shrinkage

The following method was applied to quantify the percentage kidney tissue shrinkage caused by fixation and histological procedures. The volume of removed fresh kidney was calculated by Archimedes displacement method. After tissue processing and exhaustively sectioning, the kidney volume was estimated with Cavalieri method. The kidney volume shrinkage was then calculated as follows; as described by (Tran *et al.*, 2015).

$$\text{Shrinkage} = \frac{\text{Volume before} - \text{Volume after}}{\text{Volume after}}$$

Where;

Volume before: Archimedes volume

while volume after- the Cavalieri volume

3.20.4 Determination of Volume Densities of the Kidneys

In determining the volume densities of the kidneys, Cavalieri method of point counting using the STEPanizer tool was used. The number of points on the area of interest were counted and compared with the points falling on the entire kidney and the following formula was then used as described by (Vlajković *et al.*, 2005).

$$\text{Est Vv} = \frac{P\ (\text{Part})}{P\ (\text{Ref}),}$$

Where;

Est Vv -Estimated volume density

P (part) - Number of test points falling in the structure profiles (area of interest).

P (ref) -Number of test points falling on the entire kidney (reference space)

3.21 Materials and Procedure for Acquiring Kidney Photomicrographs

a. Materials

1. Swift 3.0 digital camera (20 megapixels, SC2003)
2. BP Olympus microscope
3. A flash disk
4. Histological glass slides

b. Procedure Followed in Taking Photomicrographs

1. Histological slides were mounted on the stage of the microscope
2. The focus was adjusted until the image to be photographed was in focus
3. The field was magnified appropriately
4. Photographs of the regions was taken as viewed best under the focus of the microscope
5. Photographs were transferred to the computer by use of a USB cable

6. The photographs were then uploaded and labelled using swift 3.0 software

3.22 Statistical Data Management and Analysis

Histomorphological qualitative data was collected using photomicrographs at different magnifications using a swift 3.0 (20 megapixel) digital camera, and then exported to Adobe fireworks for qualitative analysis. Data on pregnancy and histostereological outcomes that forms the parametric data was collected using structured checklists and stereological data sheets respectively, stored and coded in excel spreadsheets windows 10, version 2016. It was then exported for analysis to SPSS program for windows version 25 for analysis (Chicago Illinois). Comparative descriptive analysis of parametric data was computed by use of ANOVA followed by Tukey's post hoc multiple comparison t-tests, while MANOVA was done to obtain main and interaction effects as well as mean difference results between pantoprazole and omeprazole. Data was expressed as mean$\pm$ standard deviation (SD) for all values, and results whose $P<.05$ were considered to be statistically significant. Parametric data was presented in form of tables, while discrete data was presented inform of graphs.

3.23 Ethical Consideration

All procedures were carried out as per laid down protocols and regulations by International Animal Research Institute (IARI) of USA as outlined by (Gomez *et al.*, 2010) and the care of laboratory animals' guidelines (Bayne, 1986). This included all procedures for animal handling during experimentation, sacrificing and harvesting of tissues. Ethical considerations included the following; the researcher had been trained on laboratory animal handling, using the appropriate number of animals, in which case 30 albino rats were used for each study medicine, handling the animals as gentle as possible so as to minimize any distress to the animals, if any. Avoidance of inflicting any pain to the animals, and the humane end points were established, which were performed as per laid down protocols (Guide for the Care and Use of Laboratory Animals ., 2010) with approval from Animal Ethics Committee University of Nairobi. Ethical approval was sought and approved by the Animal care

and use committee based in the University of Nairobi (UoN), Faculty of Veterinary medicine, Department of veterinary Anatomy and Physiology, before initiation of the study (REF: FVM BAUEC/2021/328) (Appendix I).

CHAPTER FOUR

RESULTS

4.1 Chapter Introduction

The findings of this study are presented in line with the study objectives. However, the findings of the 4th objective that was focused to evaluate whether or not the observed teratogenic effects of pantoprazole and omeprazole are time or dose dependent is integrated in the findings of the first three objectives. [*NB> some tables were big and are going beyond the margins and in some cases, they spill over from one page to the next*].

4.2 The Maternal Pregnancy and Fetal Outcomes

Objective 1: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Pantoprazole and Omeprazole Influenced the Maternal and the Fetal Pregnancy Outcomes.

In assessing how the two medicines influenced the maternal and fetal pregnancy outcomes, the findings are presented in two stages as follows:

Stage 1: The comparative findings on how the two medicines [pantoprazole and omeprazole] influenced the maternal pregnancy outcomes.

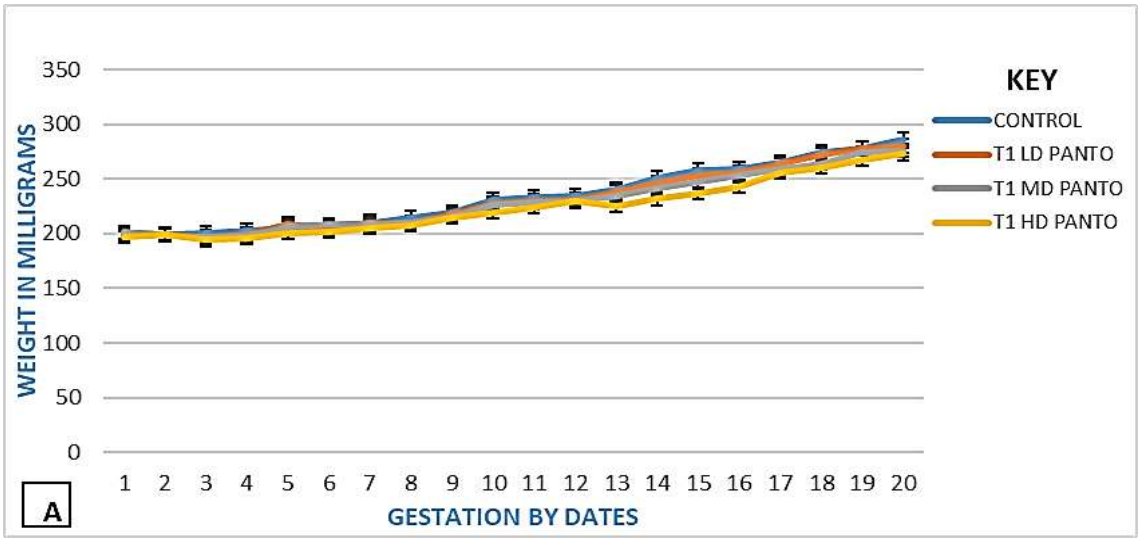
Stage 2: The comparative findings on how the two medicines influenced the fetal pregnancy outcomes.

4.2.1 The Comparative Findings on How the Two Medicines [Pantoprazole and Omeprazole] Influenced the Maternal Pregnancy Outcomes.

In evaluating how the two medicines that is, pantoprazole and omeprazole influenced the maternal pregnancy outcomes, the following parameters were evaluated; (i) the maternal weight trends, (ii) the terminal weight (iii) the mean terminal weight gain. (iv) the terminal placenta weights. This study established that, the rats in the treatment groups recorded a remarkably lower daily weight gain trends across the

entire gestation period and in all the dose groups of the low, medium and high dosages at TM_1, TM_2 and TM_3 when compared with the control. It was further noted that there was a sudden drop in daily maternal weight gain treads for one to three days then followed with sluggish increase in the means of daily weight gains trends upon initiation treatment particularly in the high and medium doses of either pantoprazole or omeprazole at different trimesters (TM_1, TM_2 and TM_3) after which the daily weight gain regained a constant but slow increase (Figure 4.1- 4.3).

Further, it was observed that the maternal weight gains depicted a dose response relationship in that; the rats that received low dosage of either drugs, had higher means of the daily maternal weight gain trends in comparison to those that received the medium and high dosages. It was also observed that when the drugs were introduced in the third trimester, the terminal weight gains did not differ significantly from the those in the control group, especially for the low and medium dosages for both the treatment groups. Upon assessing how the daily maternal weight gain treads differed between pantoprazole and omeprazole, it was noted that there was marked reduction in the weight gain trends in the omeprazole treated groups when they were compared to the pantoprazole treatment groups. The overall observation was that pantoprazole had less deleterious effects to the maternal weight gain trends compared to the omeprazole treated groups (Figure 4.1- 4.3).



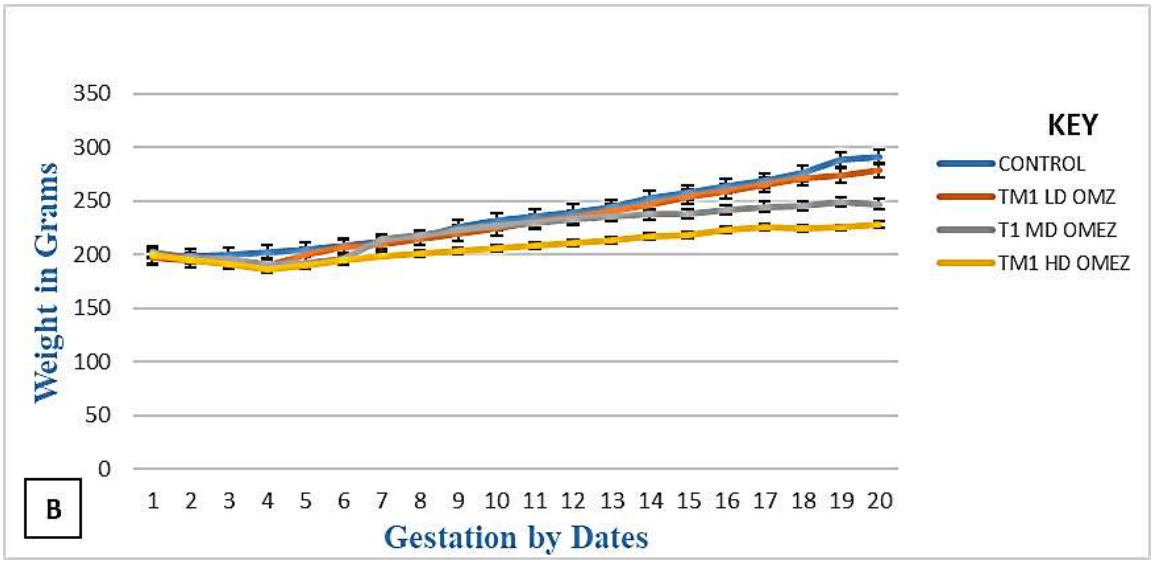


Figure 4.1: Line Graphs Showing Comparative Maternal Weight Trend for Pantoprazole and Omeprazole Treated Groups against the Control

KEY

A: Pantoprazole treated group

TM_1 LD PANTO = Trimester 1 low dose pantoprazole

TM_1 MD PANTO =Trimester 1 medium dose pantoprazole

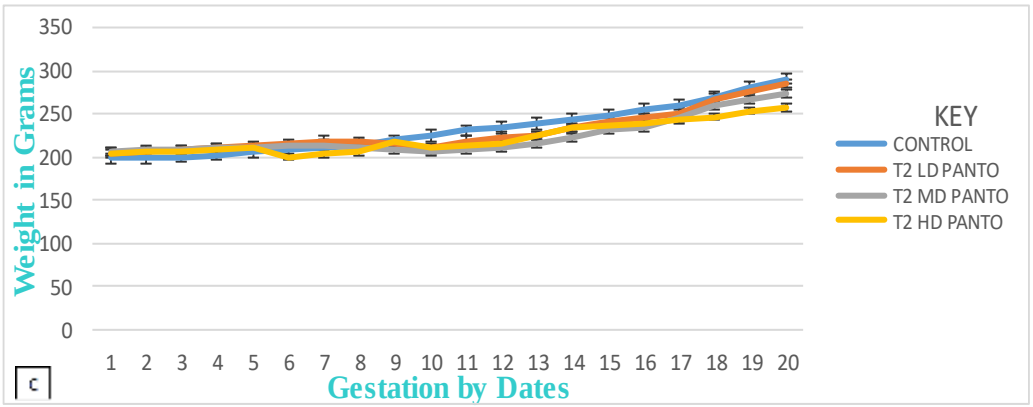
TM_1 HD PANTO = Trimester 1 high dose pantoprazole

B: Omeprazole treated group

TM_1 LD OMZ = Trimester 1 low dose omeprazole

TM_1 MD OMEZ = Trimester 1 medium dose omeprazole

TM_1 HD OMEZ = Trimester 1 high dose omeprazole



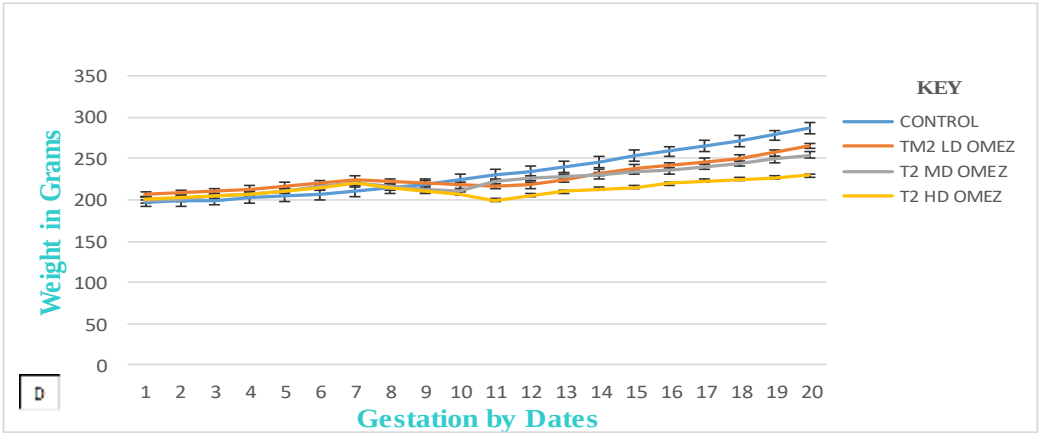


Figure 4.2: Line Graphs Showing the Comparative Daily Maternal Weight Trend for Pantoprazole and Omeprazole Treated Groups in Trimester Two against the Control

KEY:

C: Pantoprazole treated group

T2 LD PANTO = Trimester 2 low dose pantoprazole

T2 MD PANTO =Trimester 2 medium dose pantoprazole

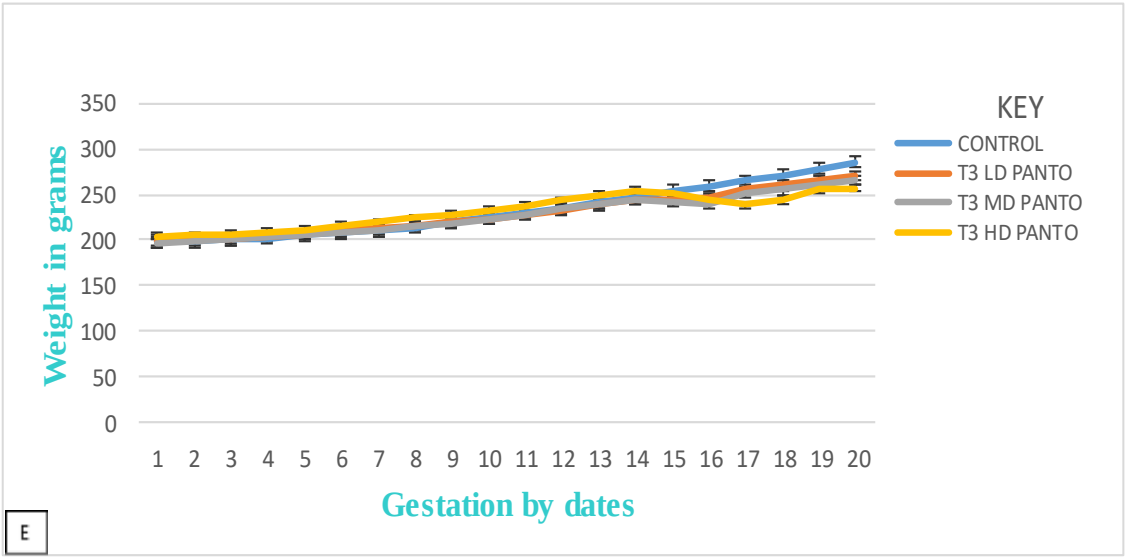
T2 HD PANTO = Trimester 2 high dose pantoprazole

D: Omeprazole treated group

TM2 LD OMEZ =Trimester 2 low dose omeprazole

T2 MD OMEZ = Trimester 2 medium dose omeprazole

T2 HD OMEZ = Trimester 2 high dose omeprazole



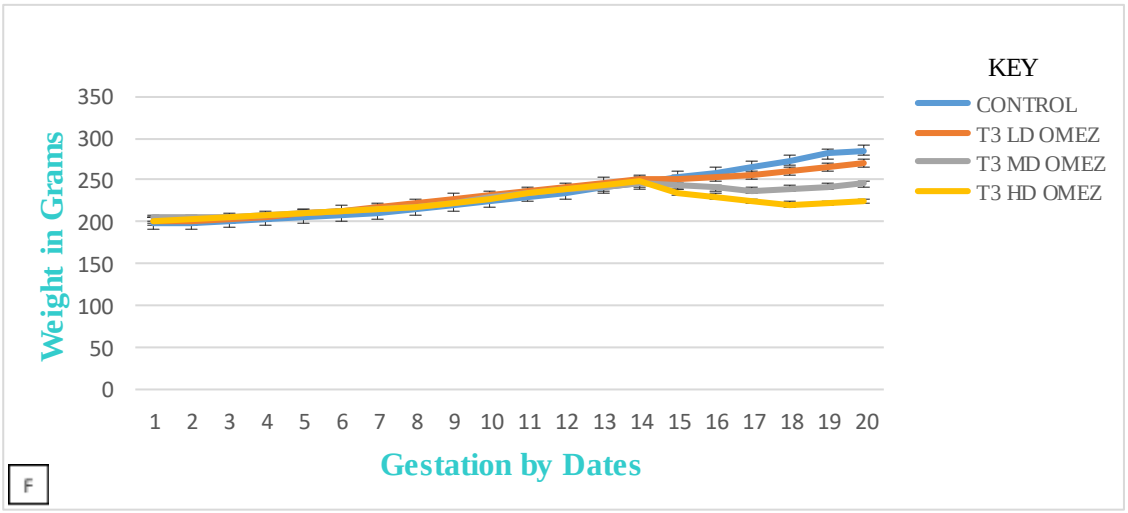


Figure 4.3: Line Graphs Showing the Comparative Daily Maternal Weight Trend for Pantoprazole and Omeprazole Treated Groups in Trimester Three against the Control

KEY

E: Pantoprazole treated group

T3 LD PANTO = Trimester 3 low dose pantoprazole

T3 MD PANTO =Trimester 3 medium dose pantoprazole

T3 HD PANTO = Trimester 3 high dose pantoprazole

F: Omeprazole treated group

TM3 LD OMEZ =Trimester 3 low dose omeprazole

TM3 MD OMEZ = Trimester 3 medium dose omeprazole

TM3 HD OMEZ = Trimester 3 high dose omeprazole

Upon assessing the overall effects of how the three independent variables of the drug, the time of exposure, and the dosages globally influenced the means of the three dependent variables of the maternal pregnancy outcomes namely; the maternal terminal weights, the mean total weight gains and the terminal placental weights, It was established that, the administration of pantoprazole and omeprazole at varied doses during pregnancy caused a statistical significant reduction (P<.05) in all the three maternal pregnancy parameters in comparison with the control as follows: (a) maternal terminal weight, (F (18,38) = 234.495, *P*=.001), (b) maternal weight gain (F (18,38) = 210.496, *P*<.001) and (c) terminal placenta weight (F (18,38) =64.748, *P*=.001 (Table 4.1, Table 4.2).

Table 4.1: The ANOVA Comparative Findings on How the Two Medicines Influenced the Means of the Three Maternal Pregnancy Outcomes Parameters at TM1, TM2 and TM3 against the Control.

The study groups	Study groups and dosage levels	The time of exposure	The comparative mean terminal weight, weight gain and placenta weight for various study groups		
			Mean terminal weight (g) ± SD	Mean weight gain (g) ± SD)	Mean placenta weight (g) ± SD)
Control.	Control (C) no treatment	None.	325.33±3.06	125.33±2.52	.490±0.01
Pantoprazole (mg/kg BW)	Low dosage group (4.13 mg/kg BW)	Trimester one	302.67±0.58	101.67±2.52	.452±0.00
		Trimester two	313.67±2.31	104.67±4.51	.470±0.01
		Trimester three	319.33±0.58	117.67±4.16	.482±0.01
	Medium dosage group (13.44 mg/kg BW)	Trimester one	287.33±.3.06*	96.33±0.58*	.434±0.01*
		Trimester two	295.33±1.15*	98.67±0.58*	.450±0.03*
		Trimester three	298.00±1.00*	101.33±1.5*	.460±0.01*
	High dosage group (24.8 mg/kg BW)	Trimester one	283.67±4.51*	89.67±2.08*	.338±0.02*
		Trimester two	288.00±1.73*	93.67±1.54*	.362±0.01*
		Trimester three	291.00±1.00*	96.67±1.53*	.398±0.02*
Omeprazole (mg/kg BW)	Low dosage group (2.07mg/kg BW)	Trimester one	275.33±1.52[b]	78.67±5.46[b]	.428±0.02[b]
		Trimester two	284.67±2.52[b]	88.33±5.32[b]	.457±0.01[b]
		Trimester three	294.00±2.00[b]	90.67±0.58[b]	.466±0.02[b]
	Medium dosage group (19.63mg/kg BW)	Trimester one	244.67±2.08*[b]	47.33±9.83*[b]	.358±0.03*[b]
		Trimester two	253.00±1.00*[b]	49.33±5.51*[b]	.415±0.01*[b]
		Trimester three	261.00±2.65*[b]	55.33±1.53*[b]	.453±0.02*[b]
	High dosage group (37.2mg/kg BW)	Trimester one	234.67±4.62*[b]	34.33±4.74*[b]	.322±0.01*[b]
		Trimester two	249.67±2.08*[b]	44.00±4.04*[b]	.349±0.01*[b]
		Trimester three	255.00±2.00*[b]	48.33±5.78*[b]	.383±0.02*[b]
Overall comparison by ANOVA (F &P) values			F (18,38) =234.495 P<0.001	F (18,38) =210.496 P<0.001	F (18,38) =64.748 P=0.001

Key: *Values are expressed as mean± standard deviation of the mean. Values that bear (*) indicates that they depict a statistical significance difference ($p<0.05$) when compared with the control, while all omeprazole values that bear (^{b}) indicates that they depict a statistical significance difference from pantoprazole ($p<0.05$) at the same dosage level using one-way ANOVA*

Upon carrying out the MANOVA level 1 analysis to determine whether multiple levels of independent variables of the drugs, dosages and the time of exposure (trimesters) plus their interactions on their own or in combination with one another had an effect on the dependent three maternal outcomes variables of the terminal weight, total weight gain and the terminal placental weights either at individual, or when combined in two way or in three ways at global level, the findings were as follows:

i. At individual level, it was observed that the drugs, doses and the time of exposure, influenced the mean reductions in the maternal pregnancy outcome parameters significantly but at different proportions as follows**; (a)** for the **drugs,** the three dependent variables of the mean terminal weight, mean maternal weight gain and mean placental weight, had statistically significant reduction with F (18, 38) ranging between 60.692 to 3194.420; P<.001 Partial Eta squared (η^2) 61.5%.to 98.8% , **(b) At dosages levels**, there was also a statistical significant reduction in the in the three maternal pregnancy outcome parameters with {F (18,2) ranging from 18.106 to 884.466; P<.001, partial Eta squared(η^2) between 48.8% to 97.9%, and **(c)** on the **time of exposure**, there was also a statistical significant reduction in the terminal maternal weight, maternal weight gain and placental weight with {F (18,2) ranging between = 51.581 to 448.512; P<.001, partial Eta squared(η^2) between 73.1% to 90.7%, as shown in the table below (table 4.2a).

ii. At two-way level of interaction effect, it was noted that the combination of (a) **drugs and the dosages** had the greatest contribution to the reduction of all the maternal outcome parameters of terminal maternal weight, weight gain and placental weight where the F(18,2) ranged between 50.258 to 370.541; P<.001, with partial Eta squared(η^2) between 72.6% to 95.1%, followed by **(b) drugs*trimesters** with F(18,2) ranging between 6.426 to 9.600; P>.05, partial Eta squared (η^2) 25.3% to 33.6% and lastly by (c) the combination **of dosages *trimesters,** where F(18,4) ranged between 1.939 to 5.668; P<.05 and partial Eta squared 21% to 24.9% respectively as shown in the (Table 4.2a) below.

iii. At three-way, the interaction effects of the three independent variables combined contributed to a lesser degree to the reduction of three maternal outcomes with F (18,4) ranging between 2.755 to 6.920; P<.05, with partial Eta squared (η^2) between 22.5% to 42.1% (Table 4.2a).

From the above, it can be deduced that, at individual level, the drugs had the highest contribution to the observed reduction in the maternal pregnancy outcomes. It can further be observed that at two-way level, the combination of drugs and dosages had highest contribution to the reduction in the maternal pregnancy outcome parameters while the three-way combination had the least contributions to the observed reductions (Table 4.2a).

Table 4.2a: The MANOVA Level II on Comparative Findings on How the Drugs, Dosages and Trimesters and their Interactions Influenced the Three Maternal Pregnancy Outcome Parameters Exposed at TM1, TM2 and TM3

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Terminal weight	36508.561[a]	18	2028.253	363.555	<.001	.994
	Weight gain	39817.474[b]	18	2212.082	237.455	<.001	.991
	Placenta weight	.150[c]	18	.008	66.939	<.001	.969
Intercept	Terminal weight	3405606.951	1	3405606.951	610438.982	<.001	1.000
	Weight gain	321380.939	1	321380.939	34498.519	<.001	.999
	Placenta weight	7.562	1	7.562	60717.171	<.001	.999
DRUGS	Terminal weight	17821.500	1	17821.500	3194.420	<.001	.988
	Weight gain	22082.667	1	22082.667	2370.456	<.001	.984
	Placenta weight	.008	1	.008	60.692	<.001	.615
DOSAGE	Terminal weight	9868.778	2	4934.389	884.466	<.001	.979
	Weight gain	8356.481	2	4178.241	448.512	<.001	.959
	Placenta weight	.005	2	.002	18.106	<.001	.488
TRIMESTER	Terminal weight	2065.333	2	1032.667	185.101	<.001	.907
	Weight gain	961.037	2	480.519	51.581	<.001	.731
	Placenta weight	.024	2	.012	97.659	<.001	.837
DRUGS * DOSAGES	Terminal weight	560.778	2	280.389	50.258	<.001	.726
	Weight gain	2289.000	2	1144.500	122.856	<.001	.866
	Placenta weight	.092	2	.046	370.541	<.001	.951
DRUGS * TRIMESTERS	Terminal weight	107.111	2	53.556	9.600	<.001	.336
	Weight gain	136.000	2	38.000	8.932	<.001	.292
	Placenta weight	.002	2	.001	6.426	.004	.253
DOSAGE * TRIMESTERS	Terminal weight	37.222	4	9.306	5.668	.008	.249
	Weight gain	100.963	4	25.241	2.709	.044	.222
	Placenta weight	.000	4	8.000	1.939	.042	.210
DRUGS * DOSAGE * TRIMESTER	Terminal weight	77.444	4	19.361	3.470	.016	.268
	Weight gain	102.667	4	25.667	2.755	.042	.225
	Placenta weight	.003	4	.001	6.920	<.001	.421
Error	Terminal weight	212.000	38	5.579			
	Weight gain	354.000	38	9.316			
	Placenta weight	.005	38	.000			
Total	Terminal weight	4566769.000	57				
	Weight gain	425410.000	57				
	Placenta weight	10.171	57				
Corrected Total	Terminal weight	36720.561	56				
	Weight gain	40171.474	56				
	Placenta weight	.155	56				

a. R Squared = .994 (Adjusted R Squared = .991)
b. R Squared = .991 (Adjusted R Squared = .987)
c. R Squared = .969 (Adjusted R Squared = .955)

Key: *(*) indicates interaction effects*

Part Two *B*: The MANOVA Pairwise Comparative Results' on How Pantoprazole and Omeprazole Influenced the Maternal Pregnancy Outcome Parameters when Exposed within the Same Dosages and the Same Trimesters.

Upon carrying out pairwise comparison on how the independent variables influenced means of the maternal pregnancy parameters of terminal weight, weight gain and placental weight, so as to establish how omeprazole compared to pantoprazole, it was observed that the means of the above dependent variables were significantly reduced in omeprazole treatment group as compared to pantoprazole treatment groups in the three trimesters as shown in the (Table 4.2b).

Table 4.2b. The MANOVA Pairwise Comparison on Maternal Pregnancy Outcome Parameters on How the Two Medicines Influenced the Three Maternal Pregnancy Outcomes when Exposed within the Same Dosage Level at the Same Trimesters

Dependent Variable	Dosage (Mg/kg BW)	Time of exposure	Drug 1	Drug 2	Mean Difference (Pantoprazole-Omeprazole)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Terminal Weight	Low	Trimester one	Pantoprazole	Omeprazole	27.333*	1.929	<.001	23.429	31.237
		Trimester two	Pantoprazole	Omeprazole	29.000*	1.929	<.001	25.096	32.904
		Trimester three	Pantoprazole	Omeprazole	25.333*	1.929	<.001	21.429	29.237
	Medium	Trimester one	Pantoprazole	Omeprazole	42.667*	1.929	<.001	38.763	46.571
		Trimester two	Pantoprazole	Omeprazole	42.333*	1.929	<.001	38.429	46.237
		Trimester three	Pantoprazole	Omeprazole	37.000*	1.929	<.001	33.096	40.904
	High	Trimester one	Pantoprazole	Omeprazole	49.000*	1.929	<.001	45.096	52.904
		Trimester two	Pantoprazole	Omeprazole	38.333*	1.929	<.001	34.429	42.237
		Trimester three	Pantoprazole	Omeprazole	36.000*	1.929	<.001	32.096	39.904
Weight Gain	Low	Trimester one	Pantoprazole	Omeprazole	23.000*	2.492	<.001	17.955	28.045
		Trimester two	Pantoprazole	Omeprazole	16.333*	2.492	<.001	11.288	21.378
		Trimester three	Pantoprazole	Omeprazole	27.000*	2.492	<.001	21.955	32.045
	Medium	Trimester one	Pantoprazole	Omeprazole	49.000*	2.492	<.001	43.955	54.045
		Trimester two	Pantoprazole	Omeprazole	49.333*	2.492	<.001	44.288	54.378
		Trimester three	Pantoprazole	Omeprazole	46.000*	2.492	<.001	40.955	51.045
	High	Trimester one	Pantoprazole	Omeprazole	55.333*	2.492	<.001	50.288	60.378
		Trimester two	Pantoprazole	Omeprazole	49.667*	2.492	<.001	44.622	54.712
		Trimester three	Pantoprazole	Omeprazole	48.333*	2.492	<.001	43.288	53.378
Placenta Weight	Low	Trimester one	Pantoprazole	Omeprazole	.091*	.009	<.001	.072	.109
		Trimester two	Pantoprazole	Omeprazole	.095*	.009	<.001	.076	.113
		Trimester three	Pantoprazole	Omeprazole	.068*	.009	<.001	.050	.087
	Medium	Trimester one	Pantoprazole	Omeprazole	.076*	.009	<.001	.058	.095
		Trimester two	Pantoprazole	Omeprazole	.034*	.009	.001	.016	.053
		Trimester three	Pantoprazole	Omeprazole	.007*	.009	.046	.012	.025
	High	Trimester one	Pantoprazole	Omeprazole	.130*	.009	<.001	.111	.148
		Trimester two	Pantoprazole	Omeprazole	.121*	.009	<.001	.103	.140
		Trimester three	Pantoprazole	Omeprazole	.098*	.009	<.001	.080	.117

Key: *(*) Means that mean difference is statistically significance at P<0.05*

4.2.2 The Comparative Findings on How the Two Medicines Influenced the Fetal Pregnancy Outcomes

The fetal pregnancy outcome parameters were assessed at two levels as follows:

Level 1: The intra-uterine fetal outcomes that included the: litter sizes, resorbed endometrial glands and the dead fetuses.

Level 2: The fetal growth and development parameters: fetal body weight and crown rump length.

4.2.2.1 The Comparative Intra-Uterine Fetal Outcome Findings for Pantoprazole and Omeprazole Treated Groups against the Control.

The intra-uterine fetal outcome parameters that were evaluated included the litter size, the resorbed endometrial glands and the dead fetuses. The litter size was observed to be highest in control group (total of 39 fetuses) than in both pantoprazole and omeprazole treatment groups (whose litter size ranged between 14-33 fetuses), (Figure 4.4A). It was also observed that in both treatment groups, the litter size was dose and time dependent in that the low and medium dosage groups had higher litter size, as compared to the high dose treatment groups. Further, the rats who received treatments during the third trimester had a higher litter size than when the two medicines were administered during the first and the second trimesters (Figure 4.4A). In addition, when the litter size was compared at the same dosage levels between the two treatment groups, it was noted to be slightly higher in pantoprazole than in omeprazole treatment group, across the three trimesters, (Figure 4.4A).

On resorbed endometrial glands, it was observed that there were no resorbed endometrial glands in the control and low dosage groups. It was however observed that in both treatment groups, the number of resorbed endometrial glands were high in the rats that were treated with high dosages of pantoprazole and omeprazole, followed by those treated with the medium dosages especially when the drugs were given from the first trimester. When the comparison was done between the two

treatment groups, the number of resorbed endometrial glands were observed to be high in the omeprazole treated groups than in pantoprazole treated groups (Figure 4.4 B).

On dead fetuses, there was no observed dead fetus in the control and low dose treatment. Contrary, the dead fetuses were observed in the treatment groups of pantoprazole and omeprazole, in which the occurrence of dead fetuses depicted a dose and time dependent relationship, in that they were observed to be high particularly in the rats that received drugs in medium and high doses as from trimesters one and two than trimester three, (Figure 4.4C).

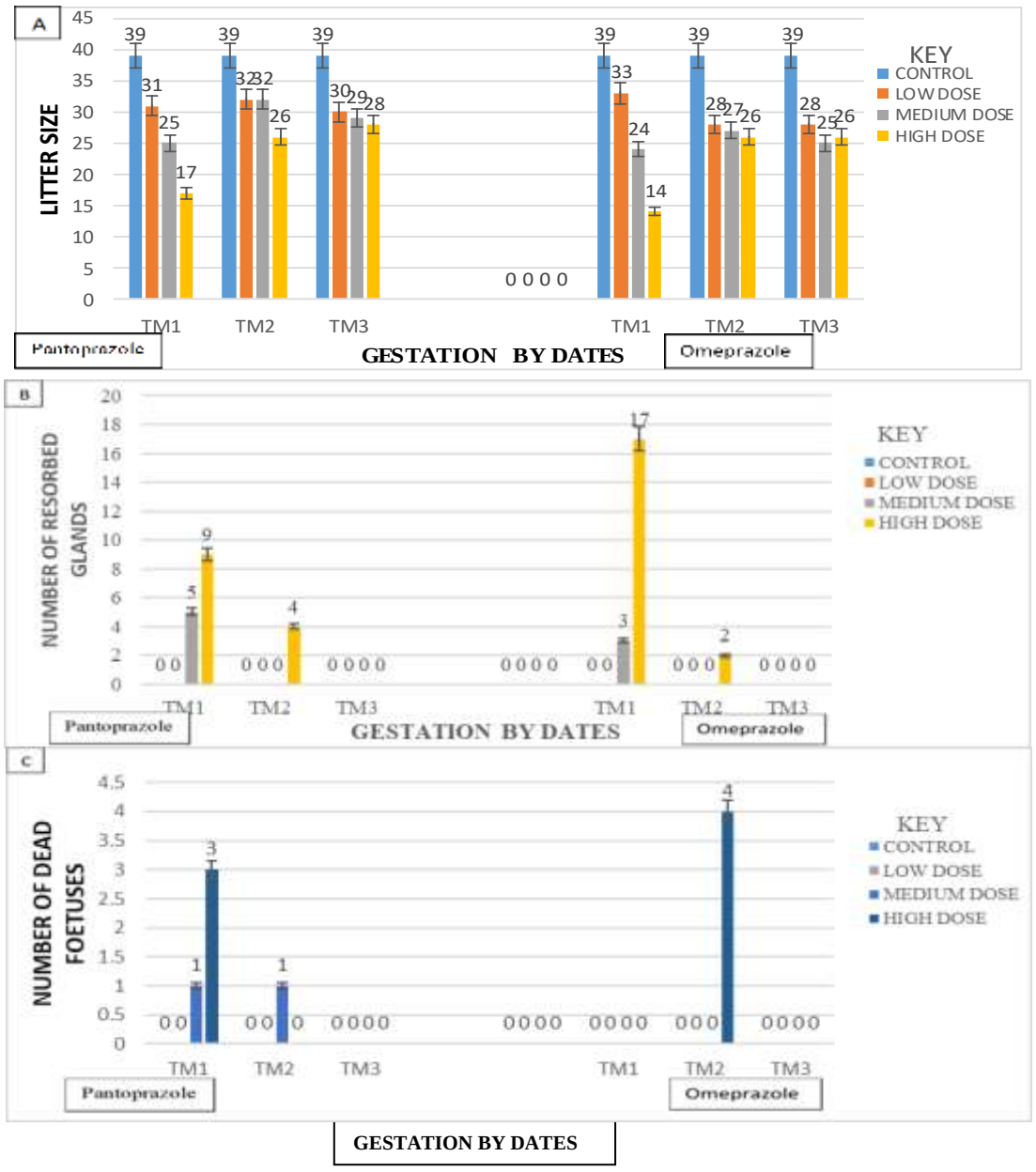


Figure 4.4: Bar Graphs Showing the Comparative Number of Litter Size, Resorbed Endometrial Glands and Dead Fetuses in Pantoprazole and Omeprazole Treated Groups against the Control.

Key

A- *the comparative litter sizes between pantoprazole and omeprazole treated groups*

B- *the comparative resorbed endometrial glands between pantoprazole and omeprazole treated groups*

C- *the comparative dead fetuses in omeprazole and pantoprazole treated groups*

Level 2: The Comparative *In-Utero* Fetal Growth and Development Parameters

In evaluating of how the two drugs influenced the fetal growth and development *in-utero*, the fetal weight and crown rump length were assessed using a univariate, bivariate and multivariate regression analysis of ANOVA and MANOVA and the results are presented in two parts as follows.

Part one: ANOVA was used to assess the overall effects of in utero exposure of the two drugs on the fetal growth parameters. It was established that, the administration of pantoprazole and omeprazole at varied doses and at different time during pregnancy caused a significant reduction($P<.05$) in the two fetal parameters when compared with the control as, indicated by the F and P values below; (a) mean fetal weight, ($F(18,38) = 168.059$, $P=<.001$) and (b) maternal weight gain ($F(18,38) = 135.031$, $P<.001$), (Table 4.3).

It was further noted that medium and high doses of omeprazole reduced the fetal growth parameters significantly more than those of pantoprazole group especially when the drugs were administered as from first and the second trimester implying that omeprazole induced in utero fetal toxicity more hence causing more detrimental effects on the fetus. However, there was no significance reduction difference when low doses of the same drugs were applied irrespective of the trimester (Table 4.3).

Table 4.3a: The ANOVA Comparative Means of Fetal Growth and Development Outcome Parameters Following Prenatal Exposure to Low, Medium and High Pantoprazole and Omeprazole at TM1, TM2, and TM3.

The study groups	Study groups and dosage levels	Duration of exposure to treatment	The comparative mean Fetal weight, and Crown rump length for various study groups	
			Mean Fetal weight (g) ± SD)	Mean CRL(g) ± SD)
Control.	Control (C) no treatment	None	6.9234±0.071	5.933±0.156
Pantoprazole (mg/kg BW)	Low dosage group (4.13mg/kg BW)	Trimester one (TM1)	6.298±0.046	5.435±0.061
		Trimester two (TM2)	6.5157±0.07	5.663±0.063
		Trimester three (TM3)	6.679±0.194	5.833±0.065
	Medium dosage group (13.44mg/kg BW)	Trimester one (TM1)	5.578±0.022*	5.202±0.161*
		Trimester two (TM2)	5.874±0.043*	5.433±0.056*
		Trimester three (TM3)	6.258±0.064*	5.601±0.134*
	High dosage group (24.8 mg/kg BW)	Trimester one (TM1)	3.948±0.442*	4.133±0.064*
		Trimester two (TM2)	4.392±0.141*	4.267±0.054*
		Trimester three (TM3)	5.504±0.070*	4.467±0.055*
Omeprazole (mg/kg BW)	Low dosage group (2.07mg/kg BW)	Trimester one (TM1)	6.144±0.032	5.233±0.122
		Trimester two (TM2)	6.380±0.065	5.367±0.062
		Trimester three (TM3)	6.487±0.144	5.433±0.113
	Medium dosage group (19.63mg/kg BW)	Trimester one (TM1)	5.283±0.041*	4.333±0.252*
		Trimester two (TM2)	5.526±0.414*	4.433±0.113*
		Trimester three (TM3)	6.084±0.095*	4.800±0.202*
	High dosage group (37.2mg/kg BW)	Trimester one (TM1)	3.624±0.163*	3.50±0.100*
		Trimester two (TM2)	4.165±0.052*	3.767±0.061*
		Trimester three (TM3)	4.969±0.116*	4.00±0.106*
Overall comparison by ANOVA (F&P) values			F (18,38) =168.059 P<0.001	F (18,38) =135.031 P<0.001

Key*: All values with (*) indicates that there is a statistical significance difference (p<0.05), when compared with the control. CRL-Crown Rump Length*

Part two: the MANOVA analysis to establish how the individual drug, dose and the time of exposure plus their interactions influenced each of the fetal growth parameters, this study found out that:

i. At individual level, it was observed that the drugs, doses and the time of exposure, influenced the fetal growth parameters significantly but at different proportions as shown by the partial Eta squared. The **drugs** had a statistical significant contribution to the reduction on two dependent variables of the fetal weight, and crown rump length, with {F (18, 38) ranging between 55.092 to 357.179; P<.001 Partial Eta squared (η^2) 59.2% to 90.4% respectively, (b) at **dosages** levels, there was also a significant contribution to the reduction in fetal weight and crown rump length with {F (18,38) ranging from 803.932 to 1070.662; P<.001, partial Eta squared(η^2) between 97.7% to 98.3% respectively, and (c) **time of exposure**, showed a statistical significant reduction in the fetal weight and crown rump length with {F (18,38) ranging between = 3.256 to 192.689; P<.05, partial Eta squared(η^2) between 14.6% to 91.0% respectively, as shown in the table below (Table 4.3b).

ii. At two-way interaction effect, it was noted that the combination of individual dosages and trimesters had the greatest contribution to the reduction in fetal weight and crown rump length where the F (18,38) ranged between 25.980 and 28.906; P<.001, with partial Eta squared(η^2) between 73.2% to 75.3%, followed by (b) drug*dosages with F (18,38) ranged between 2.671 to 31.771; P<.05, partial Eta squared (η^2) 12.3% to 62.6%. It was notable that the combination of drugs*trimesters did not have significant contribution to the reduction of the fetal weight and fetal crown lump length as shown (Table 4.3b) below.

iii. At three-way interaction effects, it was noted that the combination of all the three independent variables had a less contribution effects to the two fetal growth indicators with F (18,38) ranging between 1.324 to 2.669; P<.05, with partial Eta squared (η^2) between 12.2% to 21.9% (Table 4.3b).

From the above findings, it can be deduced that, the dosage levels of either of the drugs had the highest contribution to the observed reduction in the fetal growth parameters irrespective of the time of exposure. Secondly, the observed reductions in the fetal growth and development parameters was as a highest as a result of the combined interaction effects of dosages and trimesters, than to the combination of other independent variables. The combination of all three independent variables had a borderline contribution to the observed reduction in the fetal growth parameters.

Table 4.3b: The MANOVA Comparison on How the Drugs, Doses and Time of Exposure Plus their Interactions Influenced Each of the Two Fetal Outcome Parameters

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Fetal weight	52.019[a]	18	2.890	168.059	<.001	.988
	Crown rump length	30.275[b]	18	1.682	135.031	<.001	.985
Intercept	Fetal weight	1380.948	1	1380.948	80306.616	<.001	1.000
	Crown rump length	1040.855	1	1040.855	83561.574	<.001	1.000
Drugs	Fetal weight	.947	1	.947	55.092	<.001	.592
	Crown rump length	4.449	1	4.449	357.179	<.001	.904
Dosages	Fetal weight	36.822	2	18.411	1070.662	<.001	.983
	Crown rump length	20.028	2	10.014	803.932	<.001	.977
Trimesters	Fetal weight	6.627	2	3.313	192.689	<.001	.910
	Crown rump length	.081	2	.041	3.256	.050	.146
Drugs * Dosages	Fetal weight	.092	2	.245	2.671	.002	.123
	Crown rump length	.791	2	.396	31.771	<.001	.626
Drugs * Trimesters	Fetal weight	.009	2	.005	.273	.763	.014
	Crown rump length	.025	2	.012	.996	.379	.050
Dosages * Trimesters	Fetal weight	1.988	4	.497	28.906	<.001	.753
	Crown rump length	1.294	4	.324	25.980	<.001	.732
Drugs * Dosages * Trimesters	Fetal weight	.091	4	.123	1.324	.041	.122
	Crown rump length	.133	4	.033	2.669	.037	.219
Error	Fetal weight	.653	38	.017			
	Crown rump length	.473	38	.012			
Total	Fetal weight	1848.157	57				
	Crown rump length	1391.490	57				
Corrected Total	Fetal weight	52.672	56				
	Crown rump length	30.749	56				

a. R Squared = .988 (Adjusted R Squared = .982)

b. R Squared = .985 (Adjusted R Squared = .977)

Key*:* *The figure bearing asterisk (*) indicates there was statistical significant interaction effects*

Level 3: The MANOVA Pairwise Comparison Mean Results on How Pantoprazole and Omeprazole Influenced the Fetal Growth and Development Parameters when Exposed Within the Same Dosages and the Same Trimesters.

Upon carrying out the MANOVA pairwise comparative analysis to establish how the influence of pantoprazole and omeprazole compared on the mean fetal weight and fetal crown length when administered at the same dosage levels and at the same trimesters, it was observed that omeprazole treated groups had lower means of fetal growth parameters than pantoprazole group across the trimesters (table 4.3c).

Table 4.3c. The MANOVA Pairwise Comparison on How the Two Medicines Influenced the Means of the Two Fetal Growth Parameters when Exposed Within the Same Dosage Level, at the Same Time.

Dependent Variable	Dosage (Mg/kg bw)	Trimesters	Drug 1	Drug 2	Mean Difference (Drug 1-Drug 2)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Fetal weight	Low	Trimester one	Pantoprazole	Omeprazole	.154	.107	.159	-.063	.370
		Trimester two	Pantoprazole	Omeprazole	.135	.107	.214	-.082	.352
		Trimester three	Pantoprazole	Omeprazole	.192	.107	.081	-.025	.409
	Medium	Trimester one	Pantoprazole	Omeprazole	.295*	.107	.009	.078	.512
		Trimester two	Pantoprazole	Omeprazole	.348*	.107	.002	-.565	-.132
		Trimester three	Pantoprazole	Omeprazole	.279*	.107	.012	-.098	.391
	High	Trimester one	Pantoprazole	Omeprazole	.325*	.107	.004	.108	.542
		Trimester two	Pantoprazole	Omeprazole	.353*	.107	.041	.010	.443
		Trimester three	Pantoprazole	Omeprazole	.534*	.107	<.001	.317	.751
Crown rump length	Low	Trimester one	Pantoprazole	Omeprazole	.500*	.091	<.001	.316	.684
		Trimester two	Pantoprazole	Omeprazole	.167	.091	.015	-.018	.351
		Trimester three	Pantoprazole	Omeprazole	.233*	.091	.075	.049	.418
	Medium	Trimester one	Pantoprazole	Omeprazole	.767*	.091	<.001	.582	.951
		Trimester two	Pantoprazole	Omeprazole	1.000*	.091	<.001	.816	1.184
		Trimester three	Pantoprazole	Omeprazole	.900*	.091	<.001	.716	1.084
	High	Trimester one	Pantoprazole	Omeprazole	.633*	.091	<.001	.449	.818
		Trimester two	Pantoprazole	Omeprazole	.500*	.091	<.001	.316	.684
		Trimester three	Pantoprazole	Omeprazole	.189	.091	.083	.282	.651

Key: *(*) indicated the mean difference is statistically significant at P<0.05.*

4.3 The Histomorphological Findings

Objective 2: The Comparative Histomorphological Evaluation of How Prenatal Exposure to Varied Doses of Pantoprazole and Omeprazole Influenced the Development of the Fetal Kidneys.

The histomorphological findings of how pantoprazole and omeprazole influenced the fetal renal development is presented as follows; (a) effects in the renal corpuscles i.e. the glomerulus and bowman space, (b) distribution of the glomerulus and (c), the renal medullary and cortical thickness.

4.3.1 The Comparative Histomorphological Findings on How the Two Medicines Influenced the Histological Organization of the Glomeruli Apparatus.

In assessing how the prenatal exposure to the two medicines [pantoprazole and omeprazole] influenced the histomorphological organization of the fetal kidney glomeruli, the following histological parameters were evaluated; the glomeruli sizes, the bowman's spaces, the juxtaglomerular apparatus, and bowman's capsule. In this study, it was observed that the kidneys of fetuses of rats in the control group exhibited normal renal histomorphology that demonstrated a well outlined renal corpuscle in which the glomerulus was encased in a continuous bowman's capsule, lined by flat cells of simple squamous epithelium (Figure 4.5a). Contrary, in both treatment groups, effects in the fetal renal corpuscles were observed to depict a dose and time dependency in that, the fetuses whose mothers had been exposed to medium and high doses of both omeprazole and pantoprazole had more effects especially those that were exposed as from the first and the second trimesters (TM1&TM2).

These effects included the disrupted renal corpuscles with diminished to near complete obliteration of urinary space (bowman's space) (figure 4.5(i) &(ii) C, D, E, and figure 4.5(iii) B, C, D***)*** but in some instances, there was observed widening of the bowman's space as well as glomeruli atrophy (Figure 4.5(i), (ii) & (iii) F, G). These histomorphological changes were however more pronounced in the pantoprazole treated group in which there was noted a marked swelling/congestion of the

glomerulus with near complete obliteration of the urinary space, increased cellularity in the glomerulus, especially for rats exposed to high doses (Figure 4.5(i), (ii) (iii) B, C, D) respectively.

On the other hand, the omeprazole treated group were observed to have an increased urinary space as well as glomerular size (Figure 4.5(i), (ii) & (iii) F, G). It was further noted the kidney architecture of the fetal rats that received either of the drugs only in the last trimester, i.e., from the 15^{th} day of the gestation period were equally affected especially in the medium and high dosage group suggesting that, high doses of either omeprazole or pantoprazole affects the fetal kidneys negatively irrespective of the time of exposure (Figure 4.5(i), (ii) & (iii)).

In assessing the organization of different cells of the glomerulus, it was found that in the control group, both the macula densa and the neighboring cells of the juxta glomeruli apparatus were of normal size and demonstrated a well-organized dark staining nuclei. In addition, the cells of the nearby distal convoluted tubule were cuboidal shaped with a wide lumen and those of proximal convoluted tubules also demonstrated cuboidal shaped cells and a narrow uneven lumen. The intra-glomerular mesangial cells were also well distributed (Figure 4.5(i), (ii) & (iii) a). In contrast, it was observed that in both omeprazole and pantoprazole treatment groups, there was disruption in the arrangement of the cells with no clear distinction for the juxta glomerulus apparatus cells and macula densa cell (Figure 4.5(i), (ii) & (iii) C, D, E). This was noted more in the fetuses of the rats that received high dosages, as from at trimester one and trimester two (Figure 4.5(i) and figure 4.5 (ii) C, D, E).

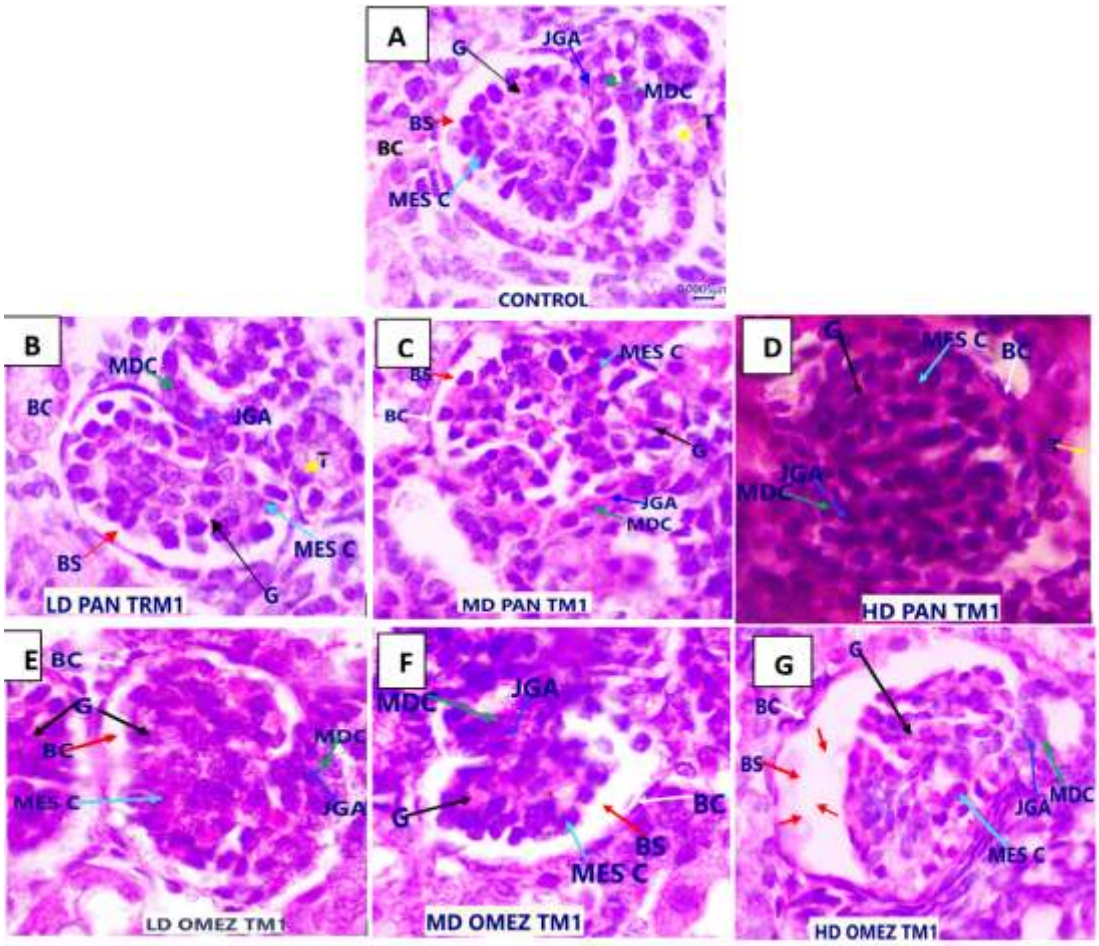


Figure 4.5(i): Photohistomicrographs Of Renal Corpuscles Showing The Comparative Changes in the Macula Densa Cells (MDC) and the Juxta Glomerulus Apparatus (JGA), the Bowman's Space (BS), Bowman's' Capsule (BC) Mesangial Cells (MES C), Tubule (T), and the Glomerulus (G), Following the Administration of Pantoprazole and Omeprazole at Different Dosages and at Trimester One against the Control (H&E X100).

Key:

Control: Shows a well outlined and sized renal corpuscle with a clear bowman's capsule lined by simple squamous epithelium

LD PAN TRM 1: Low dose pantoprazole trimester one shows mildly reduced bowman's' space

MD PAN TM1: Medium dose pantoprazole trimester one showing a swollen glomerulus with obliteration of the bowman's space

HD PAN TM1: Medium dose pantoprazole trimester one shows complete obliteration of the bowman's' space and is swollen glomerulus.

LD OMEZ TM1: Low dose omeprazole trimester one shows some increase in the bowman's space

MD OMEZ TM1: Medium dose omeprazole trimester one shows disruption of the renal corpuscle with reduced urinary space (bowman's space).

HD OMEZ TM1: High dose omeprazole trimester one shows increased bowman's space

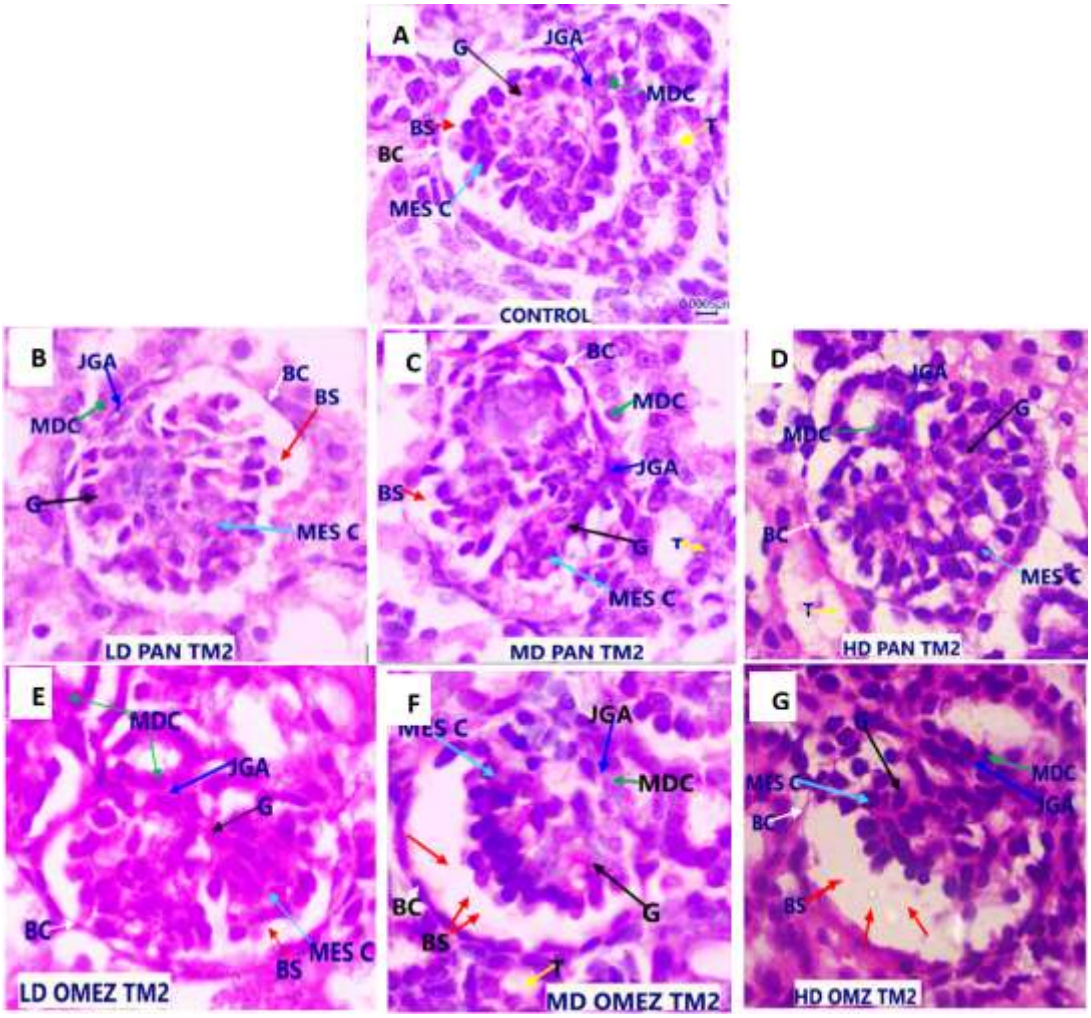


Figure 4.5(ii): Photohistomicrographs of Renal Corpuscles, Showing the Comparative Changes in the Macula Densa Cells(MDC) and the Juxta Glomerulus Apparatus (JGA), the Bowman's Space(BS), Bowman's' Capsule (BC) Mesangial Cells (MES C), Tubule (T), and the Glomerulus (G), Following the Administration of Pantoprazole and Omeprazole at Different Dosages and at Trimester Two Against the Control (H&E X100)

Key:

Control: Shows a well outlined and sized renal corpuscle with a clear bowman's capsule lined by simple squamous epithelium

LD PAN TRM 2: Low dose pantoprazole trimester two shows slightly reduced bowman's' space

MD PAN TM2: Medium dose pantoprazole trimester one showing marked reduction in bowman's space

HD PAN TM2: Medium dose pantoprazole trimester one shows an increase in the glomerulus with obliterated bowman's space.

LD OMEZ TM2: Low dose omeprazole trimester one shows a significant decrease in the bowman's space

MD OMEZ TM2: Medium dose omeprazole trimester one shows some reduction in urinary space.

HD OMEZ TM2: High dose omeprazole trimester one show atrophied glomerulus with an increased bowman's space

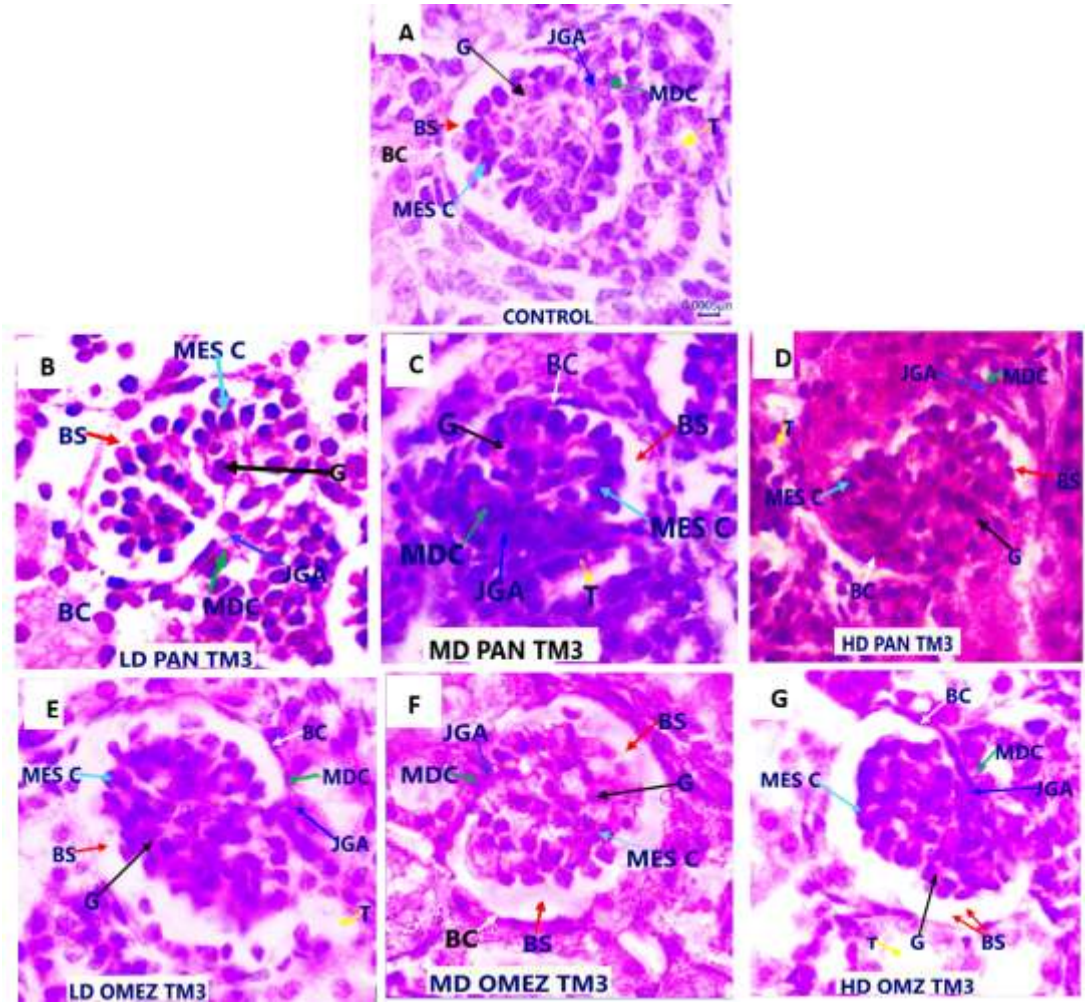


Figure 4.5(iii): Photohistomicrographs of Renal Corpuscles Showing the Comparative Changes in the Macula Densa Cells(MDC) and the Juxta Glomerulus Apparatus (JGA), the Bowman's Space(BS), Bowman's' Capsule (BC) Mesangial Cells (MES C), Tubule (T), and the Glomerulus (G), Following the Administration of Pantoprazole and Omeprazole at Different Dosages and at Trimester Three against the Control (H&E X100).

Key:

Control: Shows a well outlined and sized renal corpuscle with a clear bowman's capsule lined by simple squamous epithelium of the parietal layer

LD PAN TM 3: Low dose pantoprazole trimester three shows slightly reduction of the bowman's' space

MD PAN TM 3: Medium dose pantoprazole trimester three showing some reduction in bowman's space

HD PAN TM 3: Medium dose pantoprazole trimester three shows an increase in the glomerulus with obliterated bowman's space.

LD OMEZ TM 3: Low dose omeprazole trimester three shows a significant decrease in the bowman's space

MD OMEZ TM 3: Medium dose omeprazole trimester three shows some reduction in urinary space.

HD OMEZ TM 3: High dose omeprazole trimester three show atrophied glomerulus with an increased bowman's space

4.3.2 The Histomorphological Findings on the Distribution of the Glomerulus.

In assessing how the prenatal exposure to the two medicines [pantoprazole and omeprazole] influenced the distribution of glomeruli in the fetal kidney, it was observed that in the control group, the distribution of the fetal glomerulus per field of view was as follows, normal glomeruli which were abundant in number and well distributed. Contrary, the in the treatment groups, there was variation in the distribution of the glomerulus as shown below (figure 4.6.a, b, c). There was scanty number of glomerulus distributed per field and variation in the glomerular sizes in the treatment category that had a prolonged period of exposure to either pantoprazole or omeprazole (trimester 1and 2). Comparing the influence of the two drugs on the glomerular distribution, it was observed that omeprazole had more deleterious effects than the pantoprazole (Figures 4.6 (a), (b) & (c) E, F, G) respectively.

.

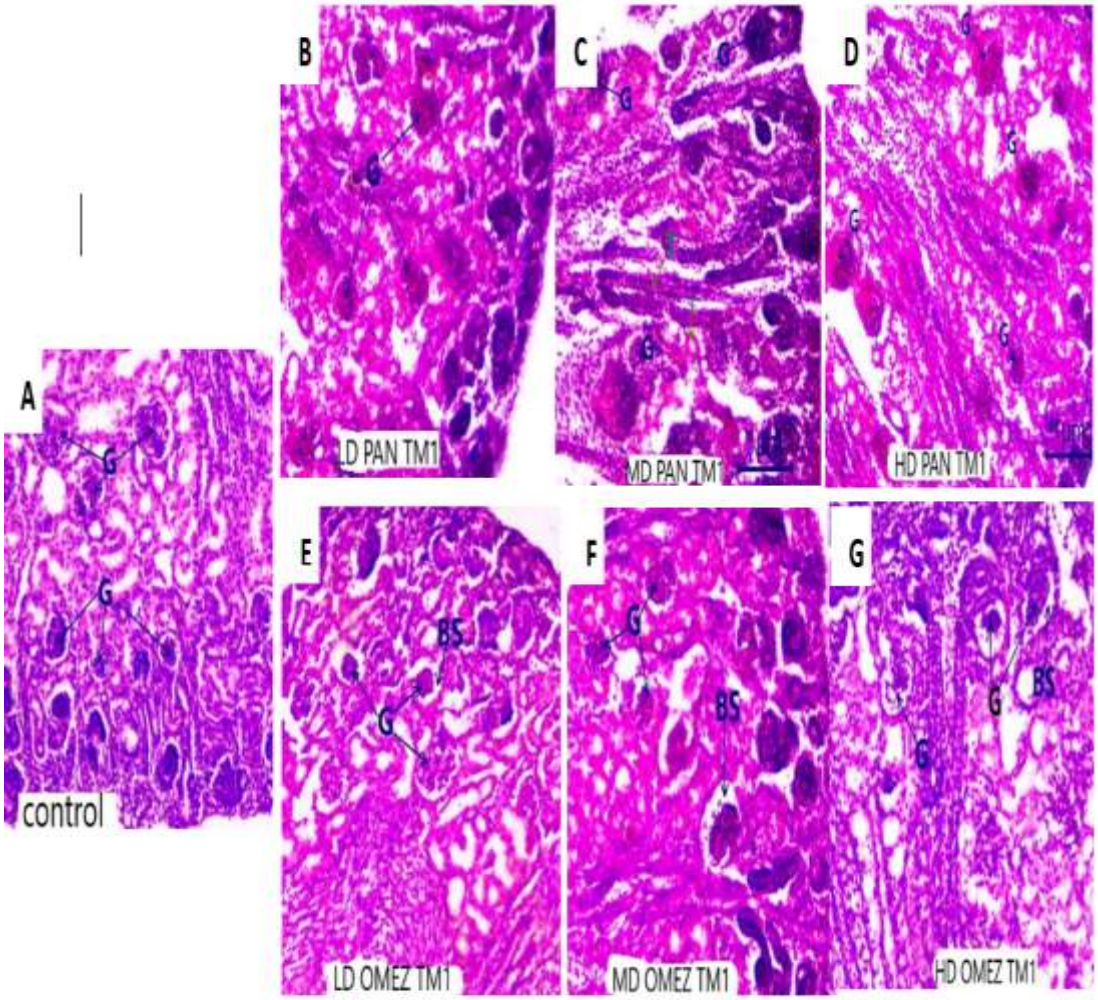


Figure 4.6(a): Histophotomicrographs Showing The Comparative Distribution Of The Glomerulus (G), Between The Pantoprazole (PAN) And Omeprazole (OMEZ) Treated Groups Of Low Dose (LD), Medium Dose (MD) And High Dose (HD) At Trimester One (TM1) Against The Control (H&E, X10)

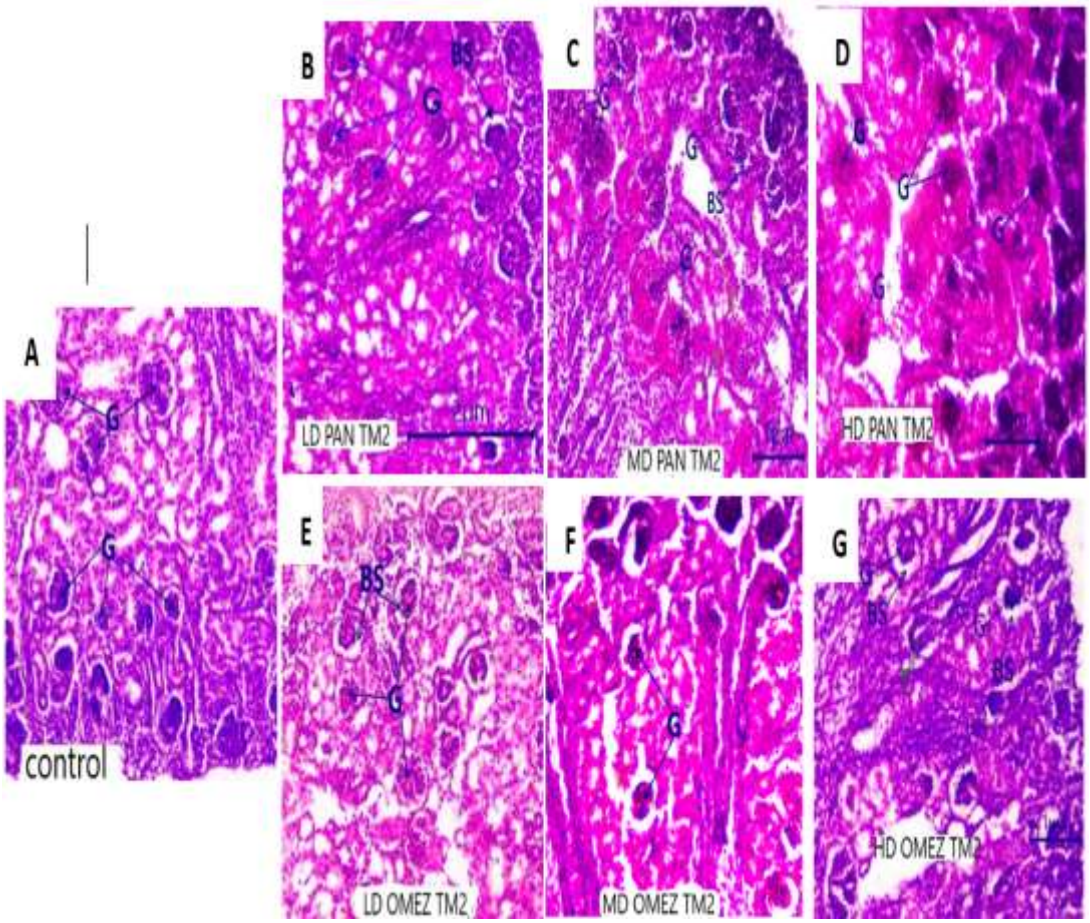


Figure 4.6(b): Histophotomicrographs Showing the Comparative Distribution of the Glomerulus (G), between the Pantoprazole (PAN) and Omeprazole (OMEZ) Treated Groups of Low Dose (LD), Medium Dose (MD) and High Dose (HD) at Trimester Two (TM2) against the Control (H&E, X10).

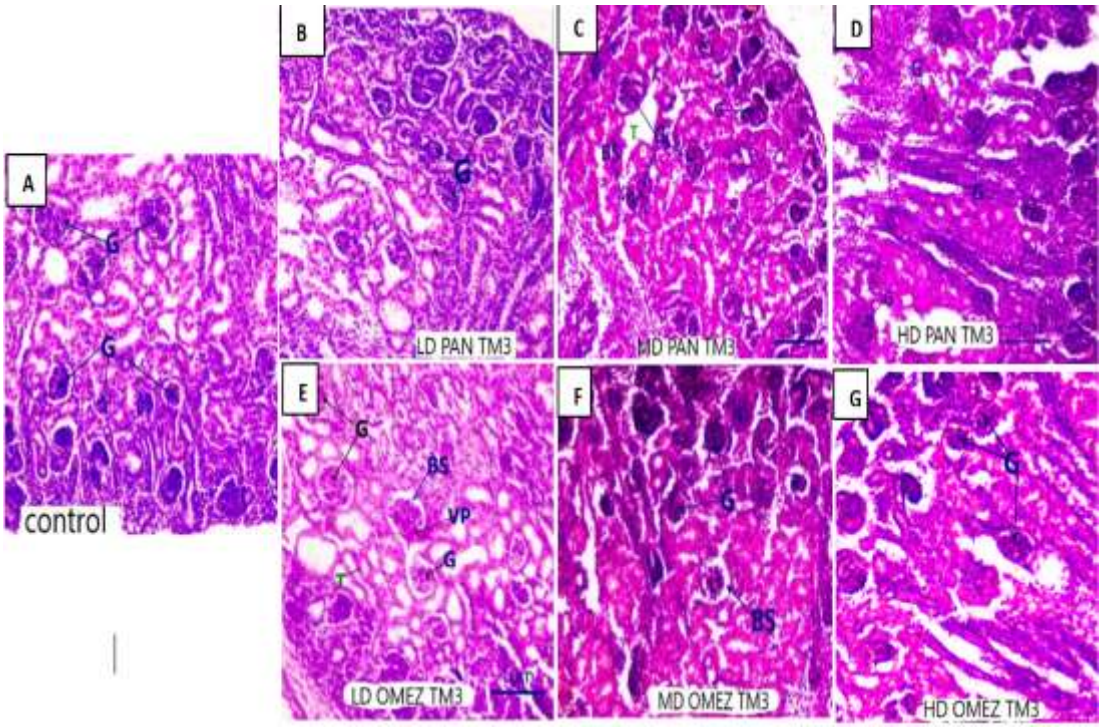


Figure 4.6(c): Histophotomicrographs Showing the Comparative Distribution of the Glomerulus (G), between the Pantoprazole (PAN) and Omeprazole (OMEZ) Treated Groups of Low Dose (LD), Medium Dose (MD) and High Dose (HD) at Trimester Three (TM3) against the Control (H&E, X10).

4.2.3. The histomorphological findings on the renal medullary and cortical thickness.

The effects of prenatal exposure to pantoprazole and omeprazole on the renal medulla and cortical thickness demonstrated an increase in the thickness of the renal medulla which showed variation across the different trimesters and was highest in high dose category for omeprazole and pantoprazole more so when the drugs were applied for prolonged duration of time (from trimester one and two) when compared to the control group category. Additionally, the cortical thickness showed a similar increase in thickness for both treatment groups when compared to the control group

category. These findings were more pronounced in the omeprazole group as shown below (figure 4.7(a, b, c).

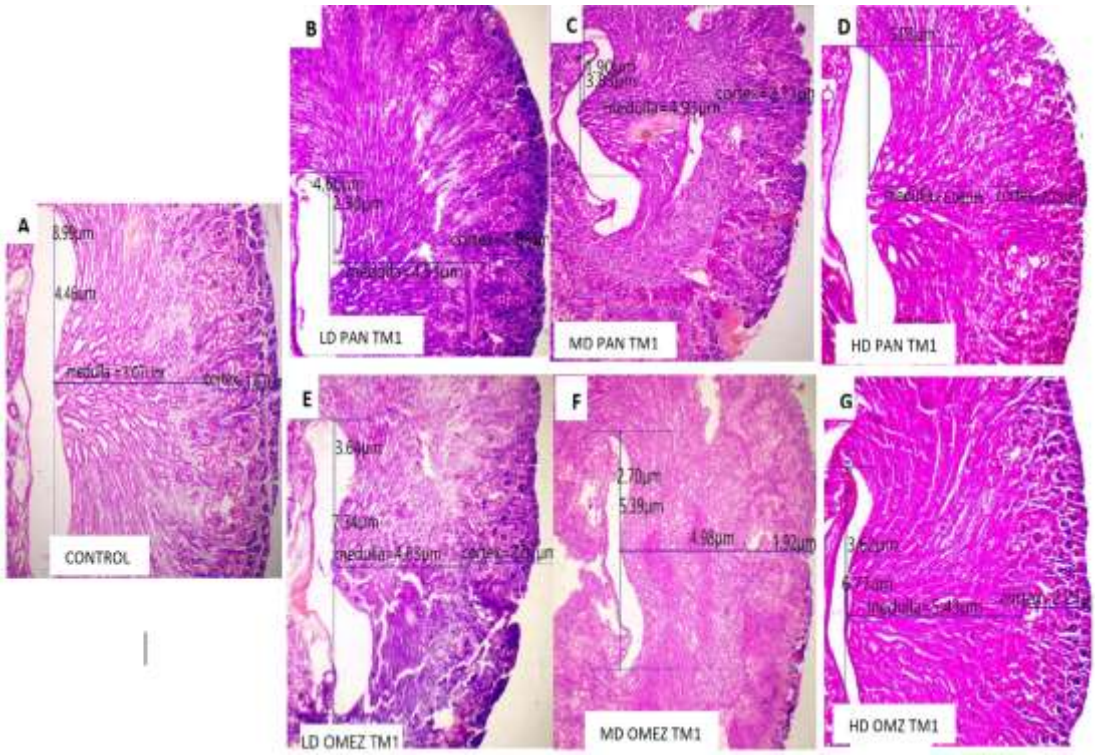


Figure 4.7(a): The Histophotomicrographs of Longitudinal Sections of the Fetal Kidneys Showing the Comparative Thicknesses of the Renal Cortex and Medulla between the Pantoprazole (PAN) and the Omeprazole (OMEZ) Treated Groups of Low (LD), Medium (MD) And High Dosages (HD), at Trimester One (TM1) against the Control (H&E, X4).

NB/ the increasing thicknesses of the medulla and the cortex

KEY:

a) *LD PAN TM1: Low dose pantoprazole trimester one*
b) *LD OMEZ TM1: Low dose omeprazole trimester one*
c) *MD PAN TM1: Medium dose pantoprazole trimester one*
d) *MD OMZ TM1Medium dose omeprazole trimester one*
e) *HD PAN TM1: High dose pantoprazole trimester one*
f) *HD OMEZ TM1: High dose omeprazole trimester one*

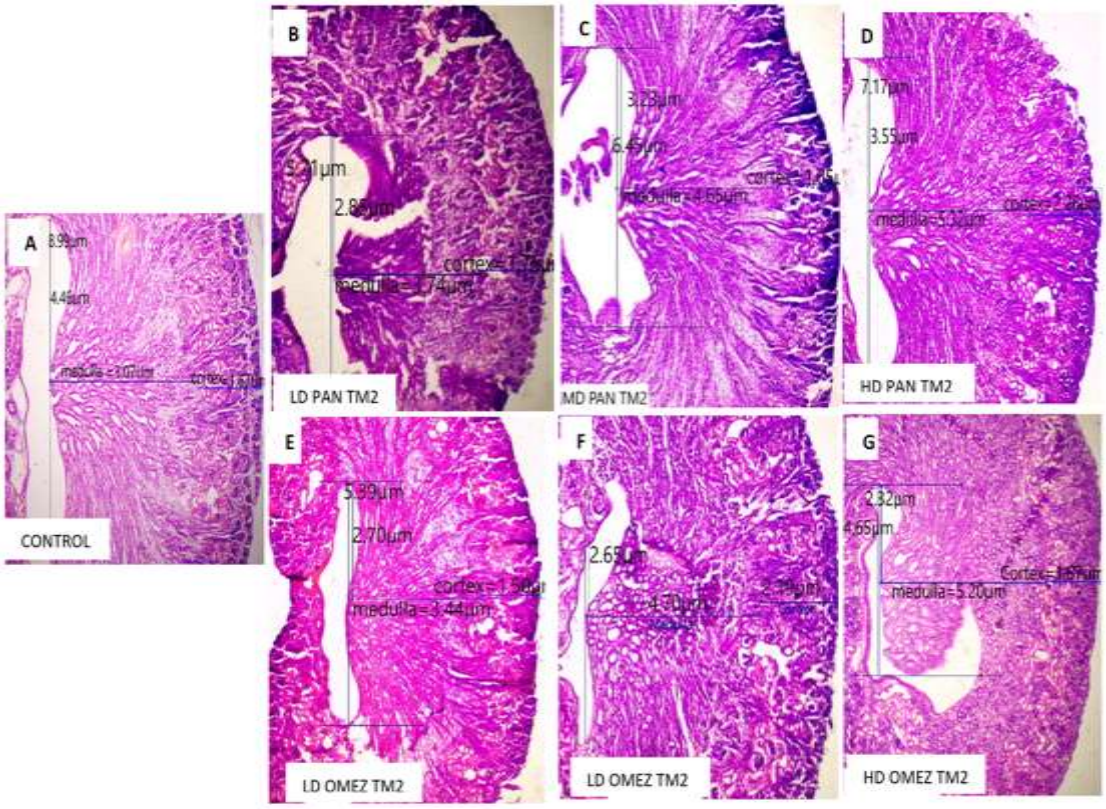


Figure 4.7(b): The Histophotomicrographs of Longitudinal Sections of the Fetal Kidneys Showing the Comparative Thicknesses of the Renal Cortex and Medulla Between the Pantoprazole (PAN) and the Omeprazole (OMEZ) Treated Groups of Low (LD), Medium (MD) and High Dosages (HD), at Trimester Two (TM2) against the Control (H&E, X4).

NB/ the increasing thicknesses of the medulla and the cortex

KEY

LD PAN TM2: Low dose pantoprazole trimester two
LD OMEZ TM2: Low dose omeprazole trimester two
MD PAN TM2: Medium dose pantoprazole trimester two
MD OMEZ TM2: Medium dose omeprazole trimester two
HD PAN TM2: High dose pantoprazole trimester two
HD OMEZ TM2: High dose omeprazole trimester two

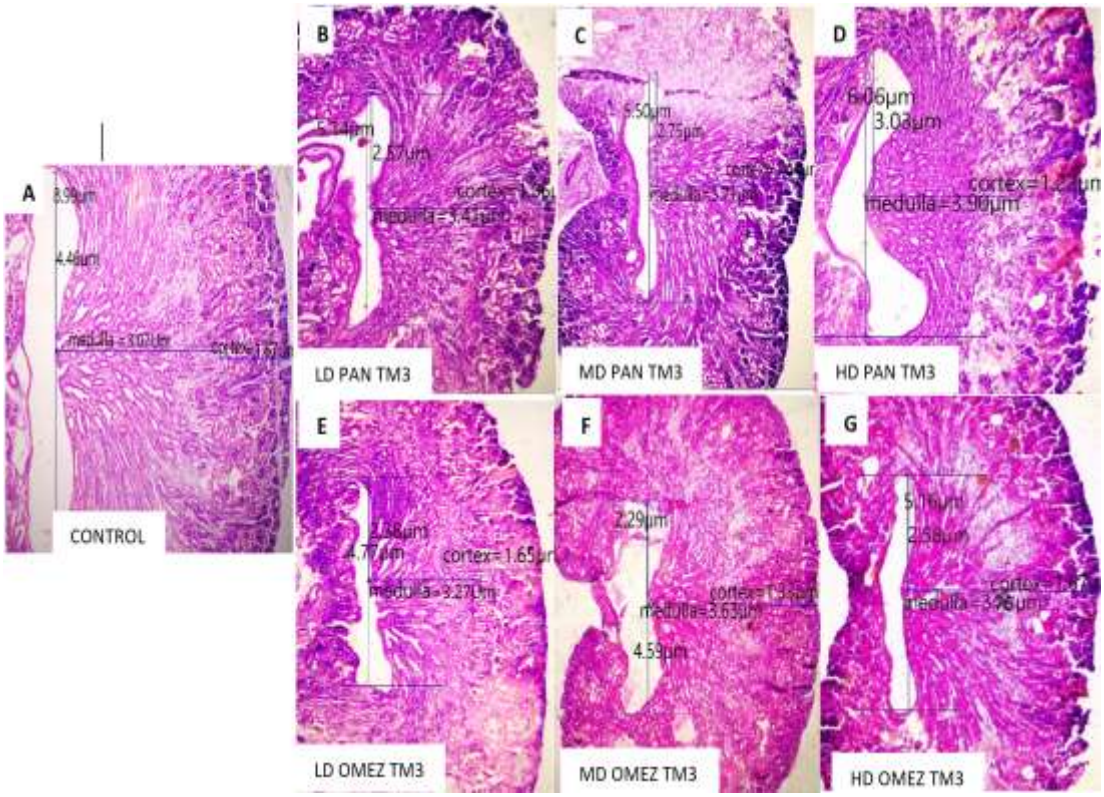


Figure 4.7(c): The Histophotomicrographs of Longitudinal Sections of the Fetal Kidneys Showing the Comparative Thicknesses of the Renal Cortex and Medulla Between the Pantoprazole (PAN) and the Omeprazole (OMEZ) Treated Groups of Low (LD), Medium (MD) and High Dosages (HD), at Trimester Three (TM3) against the Control (H&E, X4).

NB/ the increasing thicknesses of the medulla and the cortex

Key:

LD PAN TM3: Low dose pantoprazole trimester three
LD OMEZ TM3: Low dose omeprazole trimester three
MD PAN TM3: Medium dose pantoprazole trimester three
MD OMEZ TM3: Medium dose omeprazole trimester three
HD PAN TM3: High dose pantoprazole trimester three
HD OMEZ TM3: High dose omeprazole trimester three

4.4 The Histo-Stereological Findings

Objective three: the comparative stereological findings on how prenatal exposure to varied doses pantoprazole and omeprazole influenced the fetal kidney development.

The stereological findings of the prenatal exposure to pantoprazole and omeprazole at varied dosages on the fetal kidney development are presented in two levels as follows:

Level one: The comparative gross histomorphology renal findings

Level two: The comparative findings on renal histostereology

4.4.1 The ANOVA Comparative Findings on How *In-Utero* Exposure Pantoprazole and Omeprazole Influenced the Fetal Renal Gross Morphology Parameters.

In evaluating of how the two drugs and their different doses of low, medium and high dose groups within the same drug of either pantoprazole or omeprazole influenced the fetal kidney gross morphology; a univariate, bivariate and multivariate regression analysis of ANOVA and MANOVA was done and the results are presented in two parts as follows:

Part one the ANOVA analysis evaluated the overall effects of pantoprazole and omeprazole on fetal kidney gross morphology which included the means of; a) fetal kidney weight, b) fetal kidney length and c) fetal kidney width. It was established that, the administration of pantoprazole and omeprazole during pregnancy caused a significant increase P<.05) in the gross fetal renal morphology in comparison to the control (Table 4.4). When comparing the effects between the two drugs, pantoprazole was found to have more effects to the fetal renal gross morphology compared with the omeprazole as indicated by overall F and p values as follows: (a) mean fetal kidney weight, (F (18,38) = 19.058, *P*=<.001), (b) mean fetal kidney length (F (18,38) = 35.271, *P*<.001), and (c) mean fetal kidney width {F (18,38) =15.989; P<.001 respectively (Table 4.4).

In terms of dosages administered, it was notable that medium and high doses of pantoprazole caused a statistical significant increase P<.001 in the fetal renal gross morphology parameters than those of omeprazole group especially when the drugs were administered as from first and the second trimester, as shown in (Table 4.4).

Table 4.4: The ANOVA comparative means on how prenatal exposure to omeprazole and pantoprazole influenced the means of fetal renal gross morphology parameters.

The study groups	Study groups and dosage levels	Duration of exposure to treatment	The comparative means of fetal kidney weight, kidney length and kidney width for at TM1, TM2 and TM3		
			Mean kidney weight (g) ± SD)	Mean kidney length (mm) ± SD)	Mean kidney width (mm)±SD
Control.	Control (C) no treatment	None	.0459±.0001	5.770±.005	3.8667±.058
	Low dosage group	Trimester one	.04813±.005	5.790±.001	2.7233±.021
Pantoprazole	(4.13mg/kg BW)	Trimester two	.04710±.009	5.780±.005	2.8333±.058
(mg/kg BW)		Trimester three	.04559±.017	5.853±.003	3.300±.100
	Medium dosage group	Trimester one	.05822±.004*	6.147±.004*	3.564±.208*
		Trimester two	.05901±.008*	6.067±.005*	3.3333±.05*
	(13.44mg/kg BW)	Trimester three	.06012±.014*	6.113±.004*	3.5627±.05*
	High dosage group	Trimester one	.06564±.005*	6.403±002*	3.21±.102*
		Trimester two	.05909±.007*	6.32±.004*	3.4667±.05*
	(24.8 mg/kg BW)	Trimester three	.05529±.011*	6.248±.008*	3.6667±.05*
Omeprazole	Low dosage group	Trimester one	.04533±.015	5.740±.007	2.6200±.012
(mg/kg BW)	(2.07mg/kg BW)	Trimester two	.04733±.015	5.800±.008	2.7467±.040
		Trimester three	.0461±.0114	5.840±.007	2.900±.03
	Medium dosage group	Trimester one	.05417±.001*	6.043±.006*	2.7633±0.0*
		Trimester two	.0572±.1784*	5.976±.007*	3.0033±0.1*
	(19.63mg/kg BW)	Trimester three	.05801±.011*	6.167±.006*	3.3233±0.0*
	High dosage group (37.2mg/kg BW)	Trimester one	.05967±0.00*	6.100±.010*	3.9333±0.0*
		Trimester two	.05857±0.00*	6.053±.005*	3.3±0.100*
		Trimester three	.0563±0.007*	6.094±.006*	3.5533±0.0*
Overall comparison by ANOVA (F &P) values			F (18,38) = 19.058 P<0.001	F (18,38) =35.271 P<0.001	F (18,38) = 15.989 P<0.001

Key: *All values with (*) indicates that there is a statistical significance difference (p<0.05), when compared with the control.*

Part two is the MANOVA analysis on how the individual drug, dose and the time of exposure plus their interactions influenced each of the fetal kidney gross morphology parameters that included the fetal kidney weight, fetal kidney length and fetal kidney width this study found out that:

i. At individual level, it was observed that the drugs, doses and the time of exposure, influenced the gross morphology of the fetal kidney significantly but at different proportions as indicated; a) dosage levels had the highest contribution to the increase in fetal kidney weight, kidney length and kidney width with, {F (18,38) ranging from 50.249 to 228.501; P<.05, partial Eta squared(η^2) between 72.6% to 92.3%, followed by; (b) the drug at{F (18, 38) ranged between 1.890 to 35.046; P<.05, Partial Eta squared (η^2) 48.2% to 51.8%, as shown in the table below (Table 4.5i).

ii. At two-way level of interaction effect, the combination of dosages and dosage had a statistical significant increment in fetal kidney weight, and fetal kidney length and kidney width, where the F (18,38) ranged between 2.298 and 20.173; P<.05, with partial Eta squared(η^2) between 73.2% to 75.3%, followed by (b) drugs*trimesters with F (18,38) ranged between 2.671 to 6.862; P<.05, partial Eta squared (η^2) 10.3% to 26.5%, while (c) dosage and trimesters had the least contribution effect with partial Eta squared(η^2) between 9% to 31.5% (Table 4.5i).

iii. At three-way, combination of all the three independent variables had the least contribution to the observed increment in the fetal kidney parameters with partial Eta squared (η^2) ranging between 2.8% to 15.1% (Table 4.5i).

Table 4.5(i): The MANOVA's Test between- Subject Effect on How the Drugs, Doses and Time of Exposure Plus, their Interactions Influenced Each of the Three Fetal Kidney Gross Morphology Parameters.

Tests of Between-Subjects Effects							
Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Kidney Weight	.001[a]	18	7.133E-5	9.839	<.001	.823
	Kidney Length	.021[b]	18	.001	35.271	<.001	.944
	Kidney Width	2.038[c]	18	.113	15.989	<.001	.883
Intercept	Kidney Weight	.115	1	.115	15900.072	<.001	.998
	Kidney Length	14.647	1	14.647	433930.727	<.001	1.000
	Kidney Width	461.985	1	461.985	65245.588	<.001	.999
Drug	Kidney Weight	.000	1	.000	35.046	<.001	.480
	Kidney Length	.001	1	.001	40.770	<.001	.518
	Kidney Width	.013	1	.013	1.890	.047	.167
Dosage	Kidney Weight	.001	2	.000	50.249	<.001	.726
	Kidney Length	.015	2	.008	228.504	<.001	.923
	Kidney Width	1.433	2	.716	101.180	<.001	.842
Trimesters	Kidney Weight	.005	2	.215	74.960	.012	.207
	Kidney Length	.000	2	.000	3.985	.027	.173
	Kidney Width	.011	2	.005	2.763	.053	.089
Drug * Dosage	Kidney Weight	.035	2	.003	4.470	.018	.190
	Kidney Length	.001	2	.001	20.173	<.001	.515
	Kidney Width	.033	2	.016	2.298	.014	.198
Drug * Trimesters	Kidney Weight	.048	2	.024	2.176	.027	.203
	Kidney Length	.000	2	.000	4.441	.018	.189
	Kidney Width	.097	2	.049	6.862	.003	.265
Dosage * Trimesters	Kidney Weight	.006	4	0.001	7.697	.046	.009
	Kidney Length	.001	4	.000	4.361	.005	.315
	Kidney Width	.058	4	.015	2.058	.036	.293
Drug * Dosage * Trimesters	Kidney Weight	.006	4	.835	9.269	.026	.028
	Kidney Length	.000	4	1.366	1.048	.035	.099
	Kidney Width	.048	4	3.912	1.692	.017	.151
Error	Kidney Weight	.000	38	28.305			
	Kidney Length	.001	38	31.561			
	Kidney Width	.269	38	.007			
Total	Kidney Weight	.168	57				
	Kidney Length	20.652	57				
	Kidney Width	663.813	57				
Corrected Total	Kidney Weight	.002	56				
	Kidney Length	.023	56				
	Kidney Width	2.307	56				

a. R Squared = .823 (Adjusted R Squared = .740)
b. R Squared = .944 (Adjusted R Squared = .917)
c. R Squared = .883 (Adjusted R Squared = .828)

Key: *The figure bearing asterisk (*) indicates there was statistically significant interaction effects*

Further, when pairwise comparison was done between the two drugs, it observed was that omeprazole had lower means on the fetal kidney gross histomorphology parameters especially when treatment was instituted at TM1 and TM2 as shown below (Table 4.5i).

Table 4.5(ii): The MANOVA Pairwise Comparison on How the Two Drugs Influenced the Two Fetal Pregnancy Outcome Parameters when Exposed Within the Same Dosage Level at Different Trimesters.

Dependent Variable	Dosage (Mg/kg BW)	Time of exposure	Drug 1	Drug 2	Mean Difference (Drug 1-Drug 2)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Kidney weight	Low	Trimester one	Pantoprazole	Omeprazole	.000	.002	.880	-.005	.004
		Trimester two	Pantoprazole	Omeprazole	-.005	.002	.065	-.009	.000
		Trimester three	Pantoprazole	Omeprazole	.001	.002	.988	-.004	.004
	Medium	Trimester one	Pantoprazole	Omeprazole	.005*	.002	.017	-.010	-.001
		Trimester two	Pantoprazole	Omeprazole	.006*	.002	.012	.001	.010
		Trimester three	Pantoprazole	Omeprazole	.002*	.002	.007	-.006	.003
	High	Trimester one	Pantoprazole	Omeprazole	.007*	.002	.003	-.011	-.002
		Trimester two	Pantoprazole	Omeprazole	.008*	.002	<.001	-.013	-.004
		Trimester three	Pantoprazole	Omeprazole	.006*	.002	.014	-.010	-.001
Kidney Length	Low	Trimester one	Pantoprazole	Omeprazole	.005	.005	.299	-.005	.015
		Trimester two	Pantoprazole	Omeprazole	-.002	.005	.676	-.012	.008
		Trimester three	Pantoprazole	Omeprazole	.001	.005	.780	-.008	.011
	Medium	Trimester one	Pantoprazole	Omeprazole	.003*	.005	.006	.001	.020
		Trimester two	Pantoprazole	Omeprazole	.009*	.005	.002	-.001	.019
		Trimester three	Pantoprazole	Omeprazole	-.005*	.005	.008	-.015	.004
	High	Trimester one	Pantoprazole	Omeprazole	.030*	.005	<.001	.021	.040
		Trimester two	Pantoprazole	Omeprazole	.027*	.005	<.001	.017	.036
		Trimester three	Pantoprazole	Omeprazole	.016*	.005	.002	.006	.025
Kidney width	Low	Trimester one	Pantoprazole	Omeprazole	.330	.069	.540	.191	.469
		Trimester two	Pantoprazole	Omeprazole	.067	.069	.338	-.206	.072
		Trimester three	Pantoprazole	Omeprazole	.033	.069	.630	-.106	.172
	Medium	Trimester one	Pantoprazole	Omeprazole	.003*	.069	.007	-.102	.176
		Trimester two	Pantoprazole	Omeprazole	007*	.069	.001	-.186	.092
		Trimester three	Pantoprazole	Omeprazole	.004*	.069	.004	-.099	.179
	High	Trimester one	Pantoprazole	Omeprazole	.007*	.069	.002	-.062	.216
		Trimester two	Pantoprazole	Omeprazole	.043*	.069	.015	-.182	.096
		Trimester three	Pantoprazole	Omeprazole	.003*	.069	.002	-.136	.142

Key: *(*) Means that mean difference is statistically significance at P<0.05*

4.4.2 The Comparative Histostereological Findings on How the Prenatal Exposure to the Two Drugs Influenced the Fetal Kidney Volumes.

In evaluating how the two drugs and their different doses of low, medium and high dose groups within the same drug of either pantoprazole or omeprazole influenced the fetal kidney histostereological, ANOVA and MANOVA was done and the results are presented in two parts as follows.

Part one: The ANOVA univariate and bivariate analysis evaluated the overall effects of pantoprazole and omeprazole on fetal kidney histostereology parameters that included; the initial kidney volume using Archimedes' displacement method, the gold standard volume using Cavalieri point counting method, the medullary volume density and the cortical volume density and histological thicknesses that included mean medullary and cortical thickness. It was observed that, the administration of the two drugs at varied doses during pregnancy caused a statistical significant increase (P<.05), in the means of all the fetal kidney volumes and thicknesses in comparison to the control (Table 4.6). When comparing the effects between the two drugs, pantoprazole was found to have more significant effects in the increase of the means fetal kidney volumes than omeprazole, (shown by F and P values) as follows: (a) mean Archimedes' volume (F (18,38) = 17.738, P=<.001), (b) mean Cavalieri volume (F (18,38) = 13.015, P<.001), mean medulla volume density {F (18,38) =12.757; P<.001 and mean cortical volume density {F (18,38) =12.757; P<.001, medullary thickness {F(18,38) =16.717,P<.001 and cortical thickness {F(18,38) 11.555,P<.001 respectively, (Table 4.6).

Further, it was observed that medium and high doses of both pantoprazole and omeprazole led to a statistical significant increase(P=<.001) in the fetal kidney volumes when the treatments were instituted at TM1 and TM2, (Table 4.6).

Table 4.6: The ANOVA Comparative Means on How Prenatal Exposure to Pantoprazole and Omeprazole Influenced the Fetal Kidney Volumes

The study groups	Study groups and Dosage levels	Time of exposure	The comparative mean of Archimedes' volume, Cavalieri volume, medulla and cortical volume density					
			Mean Archimedes volume	Mean Cavalieri volume	Mean medulla volume density	Mean Cortical volume density	Mean medulla thickness	Mean cortical thickness
Control.	Control (C) no treatment	None	.0454±.001	0.02467±0.001	0.0164±0.001	0.008±0.001	3.107±.200	1.600±.070
Pantoprazole (mg/kg BW)	Low dosage group (4.13mg/kg BW)	Trimester I	0.0470±0.001	0.0268±0.000	0.0179±0.001	0.0087±0.001	3.990±.5153	1.687±.148
		Trimester II	0.0463±0.001	0.0278±0.002	0.0185±0.001	0.0093±0.001	3.423±.3355	1.367±.122
		Trimester III	0.0464±0.001	0.0288±0.001	0.0192±0.000	0.0096±0.000	3.650±.361	1.480±.151
	Medium dosage group (13.44mg/kg BW)	Trimester I	0.0492±0.001*	0.0267±0.001*	0.0178±0.001*	0.0089±0.001*	4.830±.537*	1.857±.237*
		Trimester II	0.0489±0.001*	0.0325±0.002*	0.0217±0.001*	0.0108±0.001*	4.543±.427*	1.700±.181*
		Trimester III	0.0475±0.001*	0.0351±0.002*	0.0234±0.001*	0.0117±0.005*	3.580±.368	1.493±.261
	High dosage group (24.8 mg/kg BW)	Trimester I	0.0501±0.002*	0.0309±0.002*	0.0206±0.001*	0.0103±0.007*	5.473±.297*	2.260±.298*
		Trimester II	0.0518±0.001*	0.0309±0.002*	0.0206±0.001*	0.0103±0.003*	5.257±.408*	1.580±.290*
		Trimester III	0.0515±0.001*	0.0311±0.002*	0.0207±0.001*	0.0104±0.005*	3.736±.545*	1.470±.205
Omeprazole (mg/kg BW)	Low dosage group (2.07mg/kg bwt)	Trimester I	0.0467±0.001	0.0240±0.002	0.0160±0.001	0.0080±0.000	4.653±.276	2.247±.369*
		Trimester II	0.0479±0.001	0.0253±0.001	0.0169±0.001	0.0084±0.000	3.624±.331	1.663±.085
		Trimester III	0.0474±0.001	0.0284±0.001	0.0169±0.001	0.0095±0.001	3.523±.214	1.587±.132
	Medium dosage group (19.63mg/kg BW)	Trimester I	0.0485±0.004*	0.0251±0.001*	0.0173±0.002*	0.0084±0.006*	5.103±.222*	2.827±.146*
		Trimester II	0.0478±0.003*	0.0252±0.001*	0.0168±0.002*	0.0084±0.003*	4.412±.382*	2.133±.182*
		Trimester III	0.0481±0.001*	0.0273±0.002*	0.0169±0.001*	0.0091±0.001*	3.934±.225	1.626±.176
	High dosage group (37.2mg/kg BW)	Trimester I	0.0490±0.003*	0.0278±0.001*	0.0170±0.001*	0.0086±0.001*	5.970±.115*	2.400±.131*
		Trimester II	0.0503±0.002*	0.0285±0.002*	0.0177±0.001*	0.0088±0.001*	5.457±.272*	2.350±.079*
		Trimester III	0.0494±0.001*	0.0286±0.001*	0.0169±0.005*	0.0085±0.001*	4.157±.233	1.583±.323
Overall comparison by ANOVA (F &P) values			F (18,38) =17.738 P<.001	F (18,38) =13.015 P<.001	F (18,38) = 12.757 P<.001	F (18,38) 12.757 P<.001	F (18,38) = 16.717 P<.001	F (18,38) =11.555 P<.001

Key: *All values with (*) indicates that there is a statistical significance difference ($p<0.05$), when compared with the control.*

Part two is the MANOVA specific statistical test to how the three independent variables plus their interactions influenced the histostereological kidney parameters that included the; (i) Cavalieri kidney volume, (ii) medullary volume densities and (iii) cortical volume densities, this study found out that:

i. At individual level, the contributions of the three independent variables to each of the fetal kidney volumes and histological kidney thicknesses was significant but at varying proportions (partial Eta η^2). with the drugs having

the highest contribution at (46.6%-87.4%) on the means of the kidney volumes (Table 4.7i).

ii. At two-way interaction effects between the combination of the drugs, dosages and time of exposure on the fetal kidney volumes, there was a statistical significant influence ($P<.05$) following the combination of (a) drugs and dosages and (b) dosages and trimesters with drugs and dosages having the highest contribution at partial Eta squared(η^2) at 78.9%, (Table 4.7i).

iii. At three-way interaction effects, there was no statistical significant contribution effect, ($P>.05$) from the combination of all the three independent variables (Table 4.7i).

From the above findings, it was summarized that the individual independent variables had its contributions to the main effects, with drugs having the highest contribution and when interaction effects of drugs and dosages were combined and not due to the combination of the three independent variables (Table 4.7i).

Table 4.7(i): The MANOVA Results on How the Individual Drug, Dose and Time of Exposure and their Interactions Influenced the Fetal Kidney Volume Parameters.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Kidney Cavalieri	.001[b]	18	.000	36.478	<.001	.964
	Medulla Volume Density	.000[d]	18	.000	16.096	<.001	.884
	Cortical Volume Density	.001[e]	18	.000	5.791	<.001	.693
	Medulla Thickness	36.820	18	2.046	16.717	<.001	.888
	Cortical Thickness	8.883	18	.493	11.555	<.001	.846
Intercept	Medulla Volume Density	.031	1	.031	47276.797	<.001	.998
	Cortical Volume Density	.014	1	.014	9254.238	<.001	.984
	Cortical Vol Density	.003	1	.003	4827.703	<.001	.938
	Medulla Thickness	707.666	1	707.666	5783.406	<.001	.993
	Cortical Thickness	133.092	1	133.092	3116.265	<.001	.988
DRUG	Kidney Cavalieri	.000	1	.000	168.369	<.001	.874
	Medulla Volume Density	.000	1	.000	79.369	<.001	.651
	Cortical Volume Density	.036	1	.036	47.369	<.001	.687
	Medulla Thickness	.928	1	.928	7.586	.009	.466
	Cortical Thickness	2.069	1	2.069	48.444	<.001	.560
DOSAGE	Kidney Cavalieri	0.047	2	.009	29.252	.001	.834
	Medulla Volume Density	.003	2	.038	6.562	.001	.687
	Cortical Volume Density	3.633E-6	2	.005	2.762	.001	.598
	Medulla Thickness	12.913	2	6.457	52.767	<.001	.735
	Cortical Thickness	.864	2	.432	10.116	<.001	.347
TRIMESTER	Kidney Cavalieri	7.919E-5	2	3.960E-5	92.407	<.001	.538
	Medulla Volume Density	3.520E-5	2	1.760E-5	31.106	.007	.492
	Cortical Volume Density	8.799E-6	2	4.400E-6	10.254	.012	.345
	Medulla Thickness	13.894	2	6.947	56.773	<.001	.749
	Cortical Thickness	4.146	2	2.073	48.535	<.001	.719
DRUGS * DOSAGE	Kidney Cavalieri	3.930E-5	2	1.965E-5	31.119	<.001	.789
	Medulla Volume Density	1.747E-5	2	8.733E-6	12.119	<.001	.567
	Cortical Volume Density	4.367E-6	2	2.183E-6	4.119	<.001	.449
	Medulla Thickness	.095	2	.047	5.388	.001	.620
	Cortical Thickness	.099	2	.050	1.163	.004	.358
DRUGS * TRIMESTER	Kidney Cavalieri	4.097E-6	2	2.048E-6	5.159	.375	.157
	Medulla Volume Density	1.821E-6	2	9.104E-7	1.159	.623	.048
	Cortical Volume Density	4.552E-7	2	2.276E-7	.256	.447	.017
	Medulla Thickness	.347	2	.174	1.419	.255	.069
	Cortical Thickness	.513	2	.256	6.005	.005	.240
DOSAGE * TRIMESTER	Kidney Cavalieri	3.932E-5	4	9.830E-6	26.563	.001	.695
	Medulla Volume Density	1.748E-5	4	4.369E-6	7.816	.001	.503
	Cortical Volume Density	4.369E-6	4	1.092E-6	4.839	.001	.426
	Medulla Thickness	3.456	4	.034	7.060	<.001	.496
	Cortical Thickness	.418	4	.104	2.444	.063	.205
DRUGS * DOSAGE * TRIMESTER	Kidney Cavalieri	3.916E-5	4	9.791E-6	9.572	.021	.368
	Medulla Volume Density	1.741E-5	4	4.351E-6	4.433	.053	.229
	Cortical Volume Density	4.351E-6	4	1.088E-6	1.864	.036	.204
	Medulla Thickness	.389	4	.097	.795	.536	.077
	Cortical Thickness	.596	4	.149	3.489	.016	.269
Error	Kidney Cavalieri	.000	38	1.767E-6			
	Medulla Volume Density	2.708	38	.073			
	Cortical Volume Density	1.434	38	.033			
	Medulla Thickness	4.650	38	.122			
	Cortical Thickness	1.623	38	.043			
Total	Kidney Cavalieri	.044	57				
	Medulla Volume Density	.020	57				
	Cortical Volume Density	.005	57				
	Medulla Thickness	202.934	57				
	Cortical Thickness	1113.884	57				
Corrected Total	Kidney Cavalieri	.001	56				
	Medulla Volume Density	.000	56				
	Cortical Volume Density	6.435E-5	56				
	Medulla Thickness	41.470	56				
	Cortical Thickness	10.506	56				

a. R Squared = .929 (Adjusted R Squared = .895)
b. R Squared = .884 (Adjusted R Squared = .829)
c. R Squared = .577 (Adjusted R Squared = .377)
d. R Squared = .884 (Adjusted R Squared = .829)
e. R Squared = .884 (Adjusted R Squared = .829)

4.4.3: The MANOVA Pairwise Comparison Results on How Pantoprazole and Omeprazole Influenced the Fetal Kidney Volumes When Administered Within the Same Dosages and at the Same Trimesters.

On further carrying out the MANOVA pairwise comparison to determine how pantoprazole and omeprazole compared in influencing the fetal kidney volumes when administered at the same dosage levels and at the same trimester, this study established that pantoprazole had more teratogenic effects that led to the increment of the fetal kidney volume and volume densities than omeprazole ***(Table 4.7ii).***

Table 4.7(ii): The MANOVA Pairwise Comparison on How Pantoprazole and Omeprazole Influenced the Fetal Kidney Volumes When Administered Within the Same Dosages and at the Same Trimesters.

Dependent Variable	Dosage (Mg/kg BW)	Trimesters	Drug 1	Drug 2	Mean Difference (drug 1-drug 2)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Cavalieri volume	Low	Trimester one	Pantoprazole	omeprazole	.003*	.001	.014	.001	.005
		Trimester two	Pantoprazole	omeprazole	.002*	.001	.027	.000	.005
		Trimester three	Pantoprazole	omeprazole	.000*	.001	.019	-.002	.003
	Medium	Trimester one	Pantoprazole	omeprazole	.002*	.001	.046	-.001	.004
		Trimester two	Pantoprazole	omeprazole	-007*	.001	.000	.005	.009
		Trimester three	Pantoprazole	omeprazole	.008*	.001	.000	.006	.010
	High	Trimester one	Pantoprazole	omeprazole	.006*	.001	.000	.004	.008
		Trimester two	Pantoprazole	omeprazole	.004*	.001	.000	.002	.007
		Trimester three	Pantoprazole	omeprazole	.006*	.001	.000	.004	.008
Medulla volume density	Low	Trimester one	Pantoprazole	omeprazole	.002*	.001	.014	.000	.003
		Trimester two	Pantoprazole	omeprazole	.002*	.001	.027	.000	.003
		Trimester three	Pantoprazole	omeprazole	.000	.001	.757	-.001	.002
	Medium	Trimester one	Pantoprazole	omeprazole	.001	.001	.041	.000	.003
		Trimester two	Pantoprazole	omeprazole	.005*	.001	.000	.003	.006
		Trimester three	Pantoprazole	omeprazole	.005*	.001	.000	.004	.007
	High	Trimester one	Pantoprazole	omeprazole	.004*	.001	.000	.003	.006
		Trimester two	Pantoprazole	omeprazole	.003*	.001	.000	.001	.004
		Trimester three	Pantoprazole	omeprazole	.004*	.001	.000	.002	.005
Cortical volume density	Low	Trimester one	Pantoprazole	omeprazole	.001*	.000	.014	.000	.002
		Trimester two	Pantoprazole	omeprazole	.001*	.000	.027	.000	.002
		Trimester three	Pantoprazole	omeprazole	.000	.000	.757	-.001	.001
	Medium	Trimester one	Pantoprazole	omeprazole	.003*	.000	.000	.000	.001
		Trimester two	Pantoprazole	omeprazole	.002*	.000	.000	.002	.003
		Trimester three	Pantoprazole	omeprazole	.001*	.000	.015	.002	.003
	High	Trimester one	Pantoprazole	omeprazole	.002*	.000	.000	.001	.003
		Trimester two	Pantoprazole	omeprazole	.001*	.000	.000	.001	.002
		Trimester three	Pantoprazole	omeprazole	.002*	.000	.000	.001	.003

Dependent Variable	Dosage (Mg/kg BW)	Trimesters	Drug 1	Drug 2	Mean Difference (drug 1-drug 2)	Std. Error	Sig[d] (<.05)	95% Confidence Interval for Difference[d] Lower Bound	Upper Bound
Medullary thickness	Low	Trimester one	Pantoprazole	omeprazole	.663	.136	.026	.085	1.242
		Trimester two	Pantoprazole	omeprazole	.200	.136	.488	-.378	.778
		Trimester three	Pantoprazole	omeprazole	-.127	.136	.660	-.705	.452
	Medium	Trimester one	Pantoprazole	omeprazole	.003*	.136	.003	.005	.852
		Trimester two	Pantoprazole	omeprazole	.120*	.136	.001	0.003	.008
		Trimester three	Pantoprazole	omeprazole	.353	.136	.224	-.225	.932
	High	Trimester one	Pantoprazole	omeprazole	.497*	.136	.090	.000	1.075
		Trimester two	Pantoprazole	omeprazole	.200*	.136	.001	.001	.002
		Trimester three	Pantoprazole	omeprazole	.420	.136	.150	-.158	.998
Cortical thickness	Low	Trimester one	Pantoprazole	omeprazole	.560	.169	.002	.218	.902
		Trimester two	Pantoprazole	omeprazole	.297	.169	.087	-.045	.638
		Trimester three	Pantoprazole	omeprazole	.107	.169	.531	-.235	.448
	Medium	Trimester one	Pantoprazole	omeprazole	.970*	.169	.000	.028	1.312
		Trimester two	Pantoprazole	omeprazole	.433*	.169	.014	.002	.775
		Trimester three	Pantoprazole	omeprazole	.133	.169	.434	-.208	.475
	High	Trimester one	Pantoprazole	omeprazole	.140*	.169	.002	.001	.482
		Trimester two	Pantoprazole	omeprazole	.770*	.169	.000	.091	1.112
		Trimester three	Pantoprazole	omeprazole	.113	.169	.046	.058	.455

Key:(*) *Means that mean difference is statistically significant at P<0.05*

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

This discussion on the current study is presented in line with the study objectives and this is also replicated in the drawing of the study conclusions and the study recommendations.

5.1 Objective 1: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Omeprazole and Pantoprazole Influenced Both the Maternal and Fetal Pregnancy Outcomes in Albino Rats

With regards to how the prenatal exposure to varied doses of two medicines, that is, omeprazole and pantoprazole comparatively influenced the maternal pregnancy outcomes, the current study established that prenatal exposure to omeprazole at various trimester lead to significant reduction ($P<.05$) in all the maternal parameters assessed when compared to the control, including (i) maternal weight gain, (ii) placental weight and (iii) terminal maternal weight. For maternal weight gain in omeprazole, for trimester one in low, medium and high dosage were as follows 78.67±5.46, 47.33±9.83, and 34.33±4.74 respectively, for trimester two in low, medium and high dosage were as follows, 88.33±5.32, 49.33±5.51, and 44.00±4.04 respectively, and for trimester three in low, medium and high dosage were as follows, 90.67±0.58, 55.33±1.53 and 48.33±5.78 respectively (Table 4.1), while in pantoprazole treated group, the mean maternal weight gain was as follows; at TM1 in the low, medium and high dosages, 101.67±2.52, 96.33±0.58, and 89.67±2.08, and respectively, for trimester two in low, medium and high dosage were as follows, 104.67±4.5, 98.67±0.58, and 93.67±1.54 respectively, while for trimester three in low, medium and high dosage were as follows, and 117.67±4.16, 101.33±1.50 and 96.67±1.53 respectively (Table 4.1). This finding concurs with a previous study by Cui *et al.,* (2001) whose reported a decrease in weight gain following a prolonged period of administration of certain PPI. This similar finding may be attributed to the usage of a similar animal models. It however contradicts the findings of Yoshikawa *et al.,* (2009) that demonstrated an increase in weight when proton pump inhibitors were administered.

Additionally, this study established that the mean placental weight was reduced in omeprazole group as follows; in trimester one, low, medium and high dosages (.428±.02, .358±0.03, and .322±0.01) respectively, in trimester two in low, medium and high doses, .457±.00, .415±.01, and .349±.01) and in trimester three low, medium and high dosages (.453±.01, .453±.02 and .383±.02) respectively while in pantoprazole group trimester one for low, medium and high dosages; (.452±.00, .434±0.01 and .338±0.02) respectively, in trimester two low, medium and high doses (.470±.01, .450±.03, and .362±.04,) and in trimester three low, medium and high dosages (.482±.01, .460±.01 and .398±.02) respectively, (Table 4.1). These findings are in concurs with those of Bahareh et al., (2017) upon administration of lansoprazole in pregnant rats. This notable reduction in the mean placental weight was possibly due to the observation that PPIs cross the placenta and inhibit some important proteins such as ATP-binding cassette, ABC, MDRI and BCRP, which are concerned with protection of fetal exposure to various harmful compounds including drugs; and are also involved in nutrient transportation in the placenta membranes' of fetal maternal barrier (Choi *et al.,* 2023; Joshi *et al.*, 2016). The inhibition of the placental proteins is associated with increased exposure of the fetus to different compounds as well as increasing toxicity to the fetus (Karttunen *et al.*, 2017). The observed overall reduction in the maternal outcome parameters was as a result of mainly the administration of drugs (98.8%), more so when they were combined with dosages (95%) followed by dosage (97.9) and then time of exposure at (90.7%), (Table 4.2a).

This study further observed a direct correlation between the reduced placental weights with what was observed on *in-utero* outcomes in regards to the fetal growth and development parameters in both the treatment groups. As such, the mean fetal weight and mean crown rump length was noted to be statistically reduced ($P<.05$) in both medium and high dosages of pantoprazole and omeprazole treated groups in comparison with the control (Table 4.3). The omeprazole treated group had lower means in the two fetal parameters as follows: (a) mean fetal weight at first trimester, at medium and high doses of omeprazole versus pantoprazole (5.283±.041 vs 5.578±.022) and (3.624±.1633 vs 3.948±.442) respectively, trimester two, medium dose and high doses (5.526±.414 vs 5.874±.043) and (4.165±.05 2 vs 4.392±.141)

respectively, and trimester three, medium and high doses (6.084±.095 vs 6.258±.064) and (4.969±.116 vs 5.504±.07) respectively, (Table 4.3). The crown rump length was as follows; trimester one at medium and high doses of omeprazole vs pantoprazole (4.333±.252 vs 5.202±.161 and 3.50±.100 vs 4.133±.064 respectively, trimester two at medium and high doses (4.433±.113 vs 5.433±.056 and 3.767±.061 vs 4.267±.054) respectively and trimester three at medium and high doses (4.8±.202 vs 5.601±.134 and 4.00±.106 vs 4.467±.055) respectively, (Table 4.3). Further, the study established that the observed reduction in the fetal growth and developmental parameters was as a result of the drug, the dose and the time of exposure administered which contributed up to 98.3% and when the drugs were combined with dosages the contribution was 95%**,** (Table 4.2a). These findings on reduction in the fetal growth parameter could be possibly due to the inhibitory effects of the omeprazole on to the placental membrane's nutrient transporter proteins. This finding agrees with that of (Diav-Citrin *et al.,* 2005; Inhibitor & Charles, 2018).

Additionally, this study further established that, the comparative means on the number of litter size per rat in both treatment groups depicted a linear dose dependent relationship in line with the period of exposure whereby the litter size per rat was significantly reduced in the trimester one (TM1) in the omeprazole group in which it ranged from (11per rat in low dose to 5 per rat in high doses), trimester two (9 per rat in low dose to 8 per rat in high dose) and trimester three (8 per rat in low dose and 9 per rat in high dose), (Figure 4.5). Similarly, the litter size per rat in the pantoprazole treated group was reduced ranging from (10 per rat in low dose to 5 fetuses per rat in high dose) in trimester one, (11fetuses per rat in low dose to 8 per rat in high dose) in trimester two and in trimester three (10 fetuses per rat in low dose to 9 per rat in high dose), (Figure 4.5). The prenatal exposure deleterious effects of pantoprazole and omeprazole was further demonstrated in the increased number of embryonic resorption (resorbed glands) which was notably high when medium and high doses of omeprazole were applied in trimester one and two as follows (17 in high dose and 3 in medium dose), (2 in high dose) respectively and in pantoprazole (9 in high dose) whereas there were no resorbed glands in the low dose and control group. This finding concurs with a study by (Alaa *et al.,* 2019) that also demonstrated resorption sites when pantoprazole at lower dosages was used

prenatally. This similar finding maybe attributed partly to the usage of similar animal models and methodology. They also concur with (Broussard & Richter, 1998), who reported disruption of the pregnancy, embryolithalities and resorped fetuses in rabbits following administration of omeprazole at higher than usual human dose implying that higher doses of PPIs are associated with fetotoxicity.

5.2 Objective 2: The Comparative Evaluation on How the Prenatal Exposure to Varied Doses of Omeprazole and Pantoprazole Influenced the Histomorphological Differentiation of the Developing Fetal Kidneys in the Albino Rats.

Concerning how the prenatal exposure to the varied doses of omeprazole and pantoprazole influenced the histological organization of the various components of the fetal kidneys in the two treatment groups as compared with the control group, this current study established that in the control fetal rats they demonstrated well developed glomerulus structures with well outlined bowman space which was well lined by both the visceral and the parietal epithelium. Conversely, prenatal exposure to omeprazole led to a notable kidney histo-architectural changes in the fetal kidneys including (i) glomerular atrophy, (ii) bowman space dilatation, (iii) tubular atrophy, Likewise, pantoprazole treated groups showed the histo-cytoarchitectural damage to the developing fetal kidneys that included the: (i) swelling of the glomerulus, (ii) obliteration of the urinary space and (iii) tubular hypertrophy (Figure 4.1). These observed changes in the developing kidney were marked when the two drugs were administered at medium and high doses more so from trimester one and two. These findings are in tandem with (Hussein et al., 2021) who reported damage to the renal structure with vascular congestion in the kidneys of adult rats, and a decline in renal function especially when omeprazole was for prolonged duration of time. These findings probably may have been attributed to fact that the kidneys serve as the main excretion pathway for the drugs of this study, and thus making it more susceptible to such injuries (Lazarus *et al.,* 2016); especially when higher doses of these drugs are administered for a prolonged period of time (Al-Hadrawy & Mahdi Al-Turfi, 2021). Additionally, the damage in the renal histo-cytoarchitexure observed in this study such as glomeruli swelling with hyper cellularity and obliteration of the urinary

space, may further be ascribed to fact that kidneys are one of the organs in the body that constantly receive a higher resting cardiac output (Mubeen *et al.,* 2016), hence these renal cells are subjected to a substantial amount of omeprazole and pantoprazole together with their accumulated metabolites over time, such as 2-mercaptobenzimidazoles especially when the PPIs are used for a prolonged duration of time and at high doses, consequently causing damage to the renal tissue (Rudler *et al*., 2018).

Comparing the effects of the two drugs on the renal histomorphology, especially considering the duration of exposure and also variation in the dosages, it was noted that these histomorphological changes varied in the two treatment groups in which in the omeprazole treated groups, there was observed more widening of the bowman's space and atrophy of the glomeruli. These findings were more pronounced when the omeprazole was administered at high and medium dosages more from trimester one (Figure 4.5). These findings concurs with those of (Al-Hadrawy & Mahdi Al-Turfi, 2021), who observed similar histomorphological changes in the kidneys when esomeprazole, a PPI in the same generation as drugs of this study was administered to the albino rats. These similar findings may be attributed to the similar methodology applied. On the other hand, in the pantoprazole treated group there was noted marked swelling of the glomerulus with hyper cellularity of intra-glomerular mesangial cells as well as complete obliteration of the urinary space, when compared with the omeprazole group. This observed alteration in the renal corpuscle may be likely due to a cell mediated immune reaction as well as the association of the proton pumps inhibitors (PPIs) with an increased risk of damage to enzyme activity and lysosomal acidification dysfunction which increases the generation of reactive oxygen species as well as exacerbating the oxidative stress (Alaa *et al.*, 2019; Yepuri *et al.*, 2016). Furthermore, PPIs have been shown to induce hypomagnesemia which has been associated with increased oxidative stress, inflammation and endothelial cell dysfunction hence the injury to the renal tissue, (Ghebremariam *et al*., 2013).

5.3 Objective 3: Comparative Histoquantitative Teratogenic Effects Of Prenatal Exposure to Varied Doses of Omeprazole and Pantoprazole on the Fetal Kidneys in the Albino Rats.

Upon carrying out a comparative histo-stereological evaluation on the teratogenic effects of the two medicines on the gross and the histological structures of the developing fetal kidneys, this current study found out that both the omeprazole and pantoprazole stereologically affected all the histological parameters that included; (a) fetal kidney weights (b) kidney length, kidney width and the stereological aspect which included the (a) Archimedes' volume, (b) Cavalieri volume, (c) cortical and medullary volume densities. It was notable that the gross morphology of the fetal kidney in terms of the kidney weight, kidney width, kidney length and was higher in the treatment exposed groups in comparison with the control group. The statistical analysis of histomorphometry demonstrated a statistical significance ($P<0.05$) increase in these means of renal gross morphology parameters (Table 4.6) in comparison to the control. This increase in the fetal gross morphology may have been due to the observed changes in the renal corpuscles such as swelling and congestion of the glomerular (figure 4.5 (a) and (b)), and the possible tubular injury resulting to the swelling of the renal tubules as was observed by (Hussein *et al.*, 2023). There was also found an increase in the size of the means of the cortical and medullary thicknesses when compared with the control group, more so when medium and high doses of the two drugs were used (Table 4.6). The increase in the cortical thickness and medullary thickness was attributable maybe to the tubular damage as well as glomerular injury in which the increased dosage and duration of exposure contributed to further damage of the kidney, a finding which is similar to that (Yepuri *et al*., 2016).

Additionally, the renal volume parameters such us the Archimedes volume, Cavalieri volume, cortical and medullary volume densities also demonstrated an increase in size which was related proportionally to the dosage of exposure (Table 4.6), implying that higher dosage and medium dosage had more effects to the kidney volume, in tandem with a previous study by (Hussein *et al.*, 2023). The increase in the cortical volume could further be explained by the concentration of the renal

corpuscle in the cortex and that there's probability of concentration of these drugs in high quantities in the cortex, which decreases progressively as they get to the medulla, this observation concurs with those of (Basile *et al.*, 2012).

5.4 Study Conclusion

From this study, the following conclusions is made:

Both pantoprazole and omeprazole were found to be relatively safe at low doses, and when used for a short period, however at medium and higher doses they were shown to cause intrauterine fetal toxicity that included embryolithalities, resorbed endometrial gland and dead fetus alongside decreased fetal growth parameters as well as negatively affecting the maternal nutrition. Both drugs induced teratogenic kidney injuries to the histological organization of the entire renal histo-architexture that included swollen and congested glomeruli, glomerular and the renal tubular atrophy when exposed to the medium and high doses. This damage to the developing fetal kidneys also increases the risks in reduction of total fetal kidney stereological volumes and volume densities of the Malpighian bodies; a recipe to future renal failure. Conclusion is further drawn that all the teratogenic effects of *in- utero* exposure to omeprazole and pantoprazole on the developing fetal kidneys is dependent on both the time of exposure as well as the dosage applied with the most critical doses being the medium and high doses when exposed at TM_2.

5.5 Recommendations.

1. The use of omeprazole and pantoprazole should be done sparingly as their usage was found to negatively affect the fetus and the nutritional status of the mother.
2. If they are to be used, the low doses should be administered for a short duration of time as high and medium doses of both were found to cause injury to renal functional unit.

3. Further teratogenic studies on the effects of both pantoprazole and omeprazole on the developing fetal kidneys in non -human primates that have close relations to humans are recommended as this would give results that are more close to humans.

REFERENCES

Al-Hadrawy, S. M. J., & Mahdi Al-Turfi, Z. S. (2021). Effects of the long-term treatment of proton pump inhibitors on the function of kidney and liver in laboratory female rats. *Archives of Razi Institute*, *76*(4), 873–881. https://doi.org/10.22092/ARI.2021.355947.1745

Alaa, M., Din, E., El, S., El, A., El, W., Abdel, S., Mostafa, R., & El-rahman, H. A. A. (2019). *Teratogenic Effects of Pantoprazole on the Pregnant Rats and Their Fetuses during Gestation*. *55*(13), 70–77.

Ali, R. A. R., Hassan, J., & Egan, L. J. (2022a). Review of recent evidence on the management of heartburn in pregnant and breastfeeding women. *BMC Gastroenterology*, *22*(1), 219. https://doi.org/10.1186/s12876-022-02287

Ali, R. A. R., Hassan, J., & Egan, L. J. (2022b). Review of recent evidence on the management of heartburn in pregnant and breastfeeding women. *BMC Gastroenterology*, *22*(1), 219. https://doi.org/10.1186/s12876-022-02287

Andersson, T., Bergstrand, R., & Cederberg, C. (1991). *Influence of acid*. 275–278.

Animals, C. for the U. of the G. for the C. and U. of L., Research, I. for L. A., Studies, D. on E. and L., & Council, N. R. (2010). *Guide for the care and use of laboratory animals: Eighth edition* (8th ed.). National Academies Press. https://doi.org/10.17226/12910

Arifin, W. N., & Zahiruddin, W. M. (2017). Sample size calculation in animal studies using resource equation approach. *Malaysian Journal of Medical Sciences*, *24*(5), 101–105. https://doi.org/10.21315/mjms2017.24.5.11

Aykan, D., & Ergun, Y. (2018). Teratogenic evaluation of drugs used by pregnant patients with gastrointestinal system diseases. *Annals of Medical Research*, *25*(4), 751. https://doi.org/10.5455/annalsmedres.2018.07.140

Bahareh, S., Mirzaei, A., Kourosh, S., & Ghazanfari. Z. (2017). *1*. Department of

Health Education, Faculty of Health, Ilam University of Medical Sciences, *Ilam, Iran 2. 4*(1), 37–44.

Basile, D. P., Anderson, M. D., & Sutton, T. A. (2012). Pathophysiology of acute kidney injury. *Comprehensive Physiology*, *2*(2), 1303–1353. https://doi.org/10.1002/cphy.c110041

Benjamin, B. (2019). Overview of laboratory animal lifestyle, care, and management: a case study of albino rats. *Journal of Applied Sciences and Environmental Management*, *23*(8), 1431. https://doi.org/10.4314/jasem.v23i8.4

Blake, J., & Rosenblum, N. D. (2014). Renal branching morphogenesis: Morphogenetic and signaling mechanisms. *Seminars in Cell and Developmental Biology*, *36*, 2–12. https://doi.org/10.1016/j.semcdb.2014.07.011

Body, C., & Christie, J. A. (2016). Gastrointestinal Diseases in Pregnancy: Nausea, Vomiting, Hyperemesis Gravidarum, Gastroesophageal Reflux Disease, Constipation, and Diarrhea. *Gastroenterology Clinics of North America*, *45*(2), 267–283. https://doi.org/10.1016/j.gtc.2016.02.005

Broussard, C. N., & Richter, J. E. (1998). Treating gastro-oesophageal reflux disease during pregnancy and lactation. What are the safest therapy options? *Drug Safety*, *19*(4), 325–337. https://doi.org/10.2165/00002018-199819040-00007

Brück, K., Stel, V. S., Fraser, S., De Goeij, M. C. M., Caskey, F., Abu-Hanna, A., & Jager, K. J. (2015). Translational research in nephrology: chronic kidney disease prevention and public health. *Clinical Kidney Journal*, *8*(6), 647–655. https://doi.org/10.1093/ckj/sfv082

Bueters, R., Bael, A., Gasthuys, E., Chen, C., Schreuder, M. F., & Frazier, K. S. (2020). Ontogeny and Cross-species Comparison of Pathways Involved in Drug Absorption, Distribution, Metabolism, and Excretion in Neonates

(Review): Kidney. *Drug Metabolism and Disposition*, *48*(5), 353–367. https://doi.org/10.1124/DMD.119.089755

Cederberg, C., Andersson, T., & Skånberg, I. (1989). Omeprazole: Pharmacokinetics and Metabolism in Man. *Scandinavian Journal of Gastroenterology*, *24*(sup166), 33–40. https://doi.org/10.3109/00365528909091241

Charan, J., & Biswas, T. (2013). How to calculate sample size for different study designs in medical research? *Indian Journal of Psychological Medicine*, *35*(2), 121–126. https://doi.org/10.4103/0253-7176.116232

Cheer, S. M., Prakash, A., Faulds, D., & Lamb, H. M. (2003). Pantoprazole: An update of its pharmacological properties and therapeutic use in the management of acid-related disorders. *Drugs*, *63*(1), 101–132. https://doi.org/10.2165/00003495-200363010-00006

Choi, A., Noh, Y., Jeong, H. E., Choi, E.-Y., Man, K. K. C., Han, J. Y., Kim, H.-S., Yon, D. K., & Shin, J.-Y. (2023). Association Between Proton Pump Inhibitor Use During Early Pregnancy and Risk of Congenital Malformations. *JAMA Network Open*, *6*(1), e2250366. https://doi.org/10.1001/jamanetworkopen.2022.50366

Choong, P. (2003). BOOK REVIEW: Book Review. *ANZ Journal of Surgery*, *73*(10), 793–793. https://doi.org/10.1046/j.1445-2197.2003.02801.x

Ckd, D. O. F., & Graded, N. (2013). Chapter 1: Definition and classification of CKD. *Kidney International Supplements*, *3*(1), 19–62. https://doi.org/10.1038/kisup.2012.64

Cruz-Orive, L. M. (1999). Precision of Cavalieri sections and slices with local errors. *Journal of Microscopy*, *193*(3), 182–198. https://doi.org/10.1046/j.1365-2818.1999.00460.x

Cui, G. L., Syversen, U., Zhao, C. M., Chen, D., & Waldum, H. L. (2001). Long-term omeprazole treatment suppresses body weight gain and bone

mineralization in young male rats. *Scandinavian Journal of Gastroenterology*, *36*(10), 1011–1015. https://doi.org/10.1080/ 003655 201750422585

Dağlı, Ü., & Kalkan, İ. H. (2017). Treatment of reflux disease during pregnancy and lactation. *The Turkish Journal of Gastroenterology : The Official Journal of Turkish Society of Gastroenterology*, *28*(Suppl 1), S53–S56. https://doi.org/10.5152/tjg.2017.14

Diav-Citrin, O., Arnon, J., Shechtman, S., Schaefer, C., Tonningen, M., Clementi, M., SANTIS, M., Robert-Gnansia, E., Valti, E., Malm, H., & Ornoy, A. (2005). The safety of proton pump inhibitors in pregnancy: A multicentre prospective controlled study. *Alimentary Pharmacology & Therapeutics*, *21*, 269–275. https://doi.org/10.1111/j.1365-2036.2005.02306.x

Dong Seok, L., Ji Won, K., Kook Lae, L., & Byeong Gwan, K. (2021). Prevalence and predictors of gastroesophageal reflux disease in pregnant women and its effects on quality of life and pregnancy outcomes. *Journal of Gynecological Research and Obstetrics*, *7*, 008–011. https://doi.org/10. 17352/jgro.000097

Elfil, M., & Negida, A. (2019). Sampling methods in clinical research; an educational review. *Archives of Academic Emergency Medicine*, *7*(1), 3–5. https://doi.org/10.22037/emergency.v5i1.15215

Fitton, A., & Wiseman, L. (1996). Pantoprazole. A review of its pharmacological properties and therapeutic use in acid-related disorders. *Drugs*, *51*(3), 460–482. https://doi.org/10.2165/00003495-199651030-00012

Frazier, K. S. (2017). Species Differences in Renal Development and Associated Developmental Nephrotoxicity. *Birth Defects Research*, *109*(16), 1243–1256. https://doi.org/10.1002/bdr2.1088

Freedberg, D. E., Kim, L. S., & Yang, Y. (2019). Adverse effects of proton pump inhibitors: evidence and plausibility. *International Journal of Molecular*

Sciences, *20*(20), 1–15.

Gerson, L. B. (2011). Proton pump inhibitors and safety during pregnancy. *Gastroenterology*, *141*(1), 389–391. https://doi.org/10.1053/j.gastro.2011.05.017

Gerson, L. B. (2012). Treatment of gastroesophageal reflux disease during pregnancy. *Gastroenterology & Hepatology*, *8*(11), 763–764.

Ghebremariam, Y. T., LePendu, P., Lee, J. C., Erlanson, D. A., Slaviero, A., Shah, N. H., Leiper, J., & Cooke, J. P. (2013). Unexpected effect of proton pump inhibitors: elevation of the cardiovascular risk factor asymmetric dimethylarginine. *Circulation*, *128*(8), 845–853. https://doi.org/10.1161/CIRCULATIONAHA.113.003602

Good, T., Schnoll-sussman, F., & Katz, P. O. (2020). P ro t o n P u m p I n h i b i t o r s. 30, 10021.

Hartman, H. A., Lai, H. L., & Patterson, L. T. (2007). Cessation of renal morphogenesis in mice. *Developmental Biology*, *310*(2), 379–387. https://doi.org/10.1016/j.ydbio.2007.08.021

Heyne, G. W., Plisch, E. H., Melberg, C. G., Sandgren, E. P., Peter, J. A., & Lipinski, R. J. (2015). A simple and reliable method for early pregnancy detection in inbred mice. *Journal of the American Association for Laboratory Animal Science*, *54*(4), 368–371.

Howden, C. W. (1991). Clinical Pharmacology of Omeprazole. *Clinical Pharmacokinetics*, *20*(1), 38–49. https://doi.org/10.2165/00003088-199120010-00003

Hussein, H. M., El-nefiawy, N. E., Farid, H., Hamid, A., Abd, M., & Moneim, E. (2021). *Downloaded from https://academic.oup.com/qjmed/article/114/Supplement_1/hcab085.002/6379122 by Jomo Kenyatta University of Agriculture user on 02 August 2023*. *114*, 2021.

Hussein, H. M., El-nefiawy, N., Hamid, H. F. A., & Moneim, M. A. (2023). *Does Omeprazole , the Proton - Pump Inhibitor , Affects the Structure of the Kidney of Male Albino Rats ? Histological and Laboratory Study*. 23–33. https://doi.org/10.4103/jmau.jmau

Inhibitor, K., & Charles, B. S. (2018). *Pantoprazole Sodium for Injection 40 mg pantoprazole / vial (pantoprazole as pantoprazole sodium sesquihydrate) Date of Revision : Submission Control No : 213002*. 1–36.

Ishizaki, T., & Horai, Y. (1999). Review article: Cytochrome P450 and the metabolism of proton pump inhibitors - emphasis on rabeprazole. *Alimentary Pharmacology and Therapeutics*, *Supplement*, *13*(3), 27–36. https://doi.org/10.1046/j.1365-2036.1999.00022.x

Jager, K. J., Kovesdy, C., Langham, R., Rosenberg, M., Jha, V., & Zoccali, C. (2019). A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases. *Nephrology Dialysis Transplantation*, *34*(11), 1803–1805. https://doi.org/10.1093/ndt/gfz174

Jewell, R. (2007). Pantoprazole. *XPharm: The Comprehensive Pharmacology Reference*, 1–5. https://doi.org/10.1016/B978-008055232-3.62361-X

Joshi, A. A., Vaidya, S. S., St-Pierre, M. V, Mikheev, A. M., Desino, K. E., Nyandege, A. N., Audus, K. L., Unadkat, J. D., & Gerk, P. M. (2016). Placental ABC Transporters: Biological Impact and Pharmaceutical Significance. *Pharmaceutical Research*, *33*(12), 2847–2878. https://doi.org/10.1007/s11095-016-2028-8

Journal, T. (1981). *Biology :*

Karttunen, V., Mohammed, A., & Vähäkangas, K. (2017). *The Significance of ABC Transporters in Human Placenta for the Exposure of Fetus to Xenobiotics* (pp. 1275–1300). https://doi.org/10.1016/B978-0-12-804239-7.00067-6

Kumar, M., Saini, M., & Parihar, L. (2022). Preformulation Studies of Pantoprazole: Fundamental Part of Formulation Design. *Saudi Journal of Medical and Pharmaceutical Sciences*, *8*(8), 370–380. https://doi.org/10.36348/sjmps.2022.v08i08.001

Kuramoto, T., Nakanishi, S., Ochiai, M., Nakagama, H., Voigt, B., & Serikawa, T. (2012). Origins of albino and hooded rats: Implications from molecular genetic analysis across modern laboratory rat strains. *PLoS ONE*, *7*(8), 1–7. https://doi.org/10.1371/journal.pone.0043059

Lalkin, A., Loebstein, R., Addis, A., Ramezani-Namin, F., Mastroiacovo, P., Mazzone, T., Vial, T., Bonati, M., & Koren, G. (1998). The safety of omeprazole during pregnancy: A multicenter prospective controlled study. *American Journal of Obstetrics and Gynecology*, *179*(3 I), 727–730. https://doi.org/10.1016/S0002-9378(98)70072-9

Law, R., Maltepe, C., Bozzo, P., & Einarson, A. (2010). Treatment of heartburn and acid reflux associated with nausea and vomiting during pregnancy. *Canadian Family Physician Medecin de Famille Canadien*, *56*(2), 143–144.

Lazarus, B., Chen, Y., Wilson, F. P., Sang, Y., Chang, A. R., Coresh, J., & Grams, M. E. (2016). Proton Pump Inhibitor Use and the Risk of Chronic Kidney Disease. *JAMA Internal Medicine*, *176*(2), 238–246. https://doi.org/10.1001/jamainternmed.2015.7193

Lee, S.-H., & Kim, J.-E. (2021). Quality by Design Applied Development of Immediate-Release Rabeprazole Sodium Dry-Coated Tablet. *Pharmaceutics*, *13*(2). https://doi.org/10.3390/pharmaceutics13020259

Li, C. M., Zhernakova, A., Engstrand, L., Wijmenga, C., & Brusselaers, N. (2020). Systematic review with meta-analysis: the risks of proton pump inhibitors during pregnancy. *Alimentary Pharmacology & Therapeutics*, *51*(4), 410–420. https://doi.org/10.1111/apt.15610

Makunts, T., Alpatty, S., Lee, K. C., Atayee, R. S., & Abagyan, R. (2019). Proton-pump inhibitor use is associated with a broad spectrum of neurological adverse events including impaired hearing, vision, and memory. *Scientific Reports*, *9*(1), 17280. https://doi.org/10.1038/s41598-019-53622-3

Malfertheiner, S. F., Malfertheiner, M. V, Kropf, S., Costa, S.-D., & Malfertheiner, P. (2012). A prospective longitudinal cohort study: evolution of GERD symptoms during the course of pregnancy. *BMC Gastroenterology*, *12*, 131. https://doi.org/10.1186/1471-230X-12-131

Mathews, S., Reid, A., Tian, C., & Cai, Q. (2010). An update on the use of pantoprazole as a treatment for gastroesophageal reflux disease. *Clinical and Experimental Gastroenterology*, *3*(1), 11–16. https://doi.org/10.2147/ceg.s6355

Matok, I., Levy, A., Wiznitzer, A., Uziel, E., Koren, G., & Gorodischer, R. (2012). The safety of fetal exposure to proton-pump inhibitors during pregnancy. *Digestive Diseases and Sciences*, *57*(3), 699–705. https://doi.org/10.1007/s10620-011-1940-3

Mohazzab, P. (2017). Archimedes' Principle Revisited. *Journal of Applied Mathematics and Physics*, *05*(04), 836–843. https://doi.org/10.4236/jamp.2017.54073

Mubeen, A., Javed, M., & Manzoor, N. (2016). Nephrotoxic effects of omeprazole i on renal vasculature of albino wister rats by histopathological study. *Medical Forum Monthly*, *27*(8), 6–9.

Nair, A., Morsy, M. A., & Jacob, S. (2018). Dose translation between laboratory animals and human in preclinical and clinical phases of drug development. *Drug Development Research*, *79*(8), 373–382. https://doi.org/10.1002/ddr.21461

Nava-Ocampo, A. A., Velázquez-Armenta, E. Y., Han, J.-Y., & Koren, G. (2006).

Use of proton pump inhibitors during pregnancy and breastfeeding. *Canadian Family Physician Medecin de Famille Canadien*, *52*(7), 853–854.

Ochoa, D., Román, M., Cabaleiro, T., Saiz-Rodríguez, M., Mejía, G., & Abad-Santos, F. (2020). Effect of food on the pharmacokinetics of omeprazole, pantoprazole and rabeprazole. *BMC Pharmacology & Toxicology*, *21*(1), 54. https://doi.org/10.1186/s40360-020-00433-2

Park, G.-J., Bae, S. H., Park, W.-S., Han, S., Park, M.-H., Shin, S.-H., Shin, Y. G., & Yim, D.-S. (2017). Drug-drug interaction of microdose and regular-dose omeprazole with a CYP2C19 inhibitor and inducer. *Drug Design, Development and Therapy*, *11*, 1043–1053. https://doi.org/10.2147/DDDT.S131797

Pasternak, B., & Hviid, A. (2011). Use of proton-pump inhibitors in early pregnancy and the risk of birth defects. *Obstetrical and Gynecological Survey*, *66*(4), 195–197. https://doi.org/10.1097/OGX.0b013e318210f5e3

Paz, M. F. C. J., De Alencar, M. V. O. B., De Lima, R. M. I. P., Sobral, A. L. P., Do Nascimento, G. T. M., Dos Reis, C. A., Coêlho, M. D. P. S. D. S., … & Melo Cavalcante, A. A. D. C. (2020). Pharmacological Effects and Toxicogenetic Impacts of Omeprazole: Genomic Instability and Cancer. *Oxidative Medicine and Cellular Longevity*, *2020*. https://doi.org/10.1155/2020/3457890

Peron, A., Ripoche, E., Picot, C., Ajiji, P., Cucherat, M., & Cottin, J. (2023). Use of proton pump inhibitors during pregnancy: A systematic review and meta-analysis of congenital malformations. *Reproductive Toxicology*, *119*, 108419. https://doi.org/https://doi.org/10.1016/j.reprotox.2023.108419

Pharmacokinetics. (2004). Methods and Principles in Medicinal Chemistry Pharmacokinetics and Metabolism in Drug Design Drug Bioavailability Protein-Ligand Interactions Bioinformatics – From Genomes to Drugs

Protein Crystallography in Drug Discovery Drug-Membrane Interactions BioNM. In *Textbook*.

Reidy, K. J., & Rosenblum, N. D. (2009). Cell and molecular biology of kidney development. *Seminars in Nephrology*, *29*(4), 321–337. https://doi.org/ 10.1016/ j.semnephrol.2009.03.009

Robinson, M., & Horn, J. (2003). Clinical pharmacology of proton pump inhibitors: what the practising physician needs to know. *Drugs*, *63*(24), 2739–2754. https://doi.org/10.2165/00003495-200363240-00004

Rosenblum, S., Pal, A., & Reidy, K. (2017). Renal development in the fetus and premature infant. *Seminars in Fetal and Neonatal Medicine*, *22*(2), 58–66. https://doi.org/10.1016/j.siny.2017.01.001

Rudler, M., Isnard Bagnis, C., & Rudler, H. (2018). Proton Pump Inhibitors and Chronic Kidney Disease: Is It Related to the Accumulation of Toxic Breakdown Products Spontaneously Formed in the Enteric-Protected Tablets? *Gastroenterology*, *154*(4), 1204–1205. https://doi.org/10.1053/ j.gastro.2017.07.055

Sampathkumar, K., Ramalingam, R., Prabakar, A., & Abraham, A. (2013). Acute interstitial nephritis due to proton pump inhibitors. *Indian Journal of Nephrology*, *23*(4), 304–307. https://doi.org/10.4103/0971-4065.114487

Schoenfeld, A. J., & Grady, D. (2016). Adverse effects associated with proton pump inhibitors. *JAMA Internal Medicine*, *176*(2), 172–174. https://doi.org/10. 1001/jamainternmed.2015.7927

Seely, J. C. (2017). A brief review of kidney development, maturation, developmental abnormalities, and drug toxicity: Juvenile animal relevancy. *Journal of Toxicologic Pathology*, *30*(2), 125–133. https://doi.org/10.1293/tox.2017-0006

Seikaly, M. G., Ho, P. L., Emmett, L., Fine, R. N., & Tejani, A. (2003). Chronic

renal insufficiency in children: the 2001 Annual Report of the NAPRTCS. *Pediatric Nephrology (Berlin, Germany)*, *18*(8), 796–804. https://doi.org/10.1007/s00467-003-1158-5

Shah, D. A., Patel, A., Baldania, S. L., Chhalotiya, U. K., & Bhatt, K. K. (2013). *Simultaneous Estimation of Pantoprazole Sodium and Levosulpiride in Capsule Dosage Form by Simultaneous Equation Spectrophotometric Method*. *2013*.

Shanks, N., Greek, R., & Greek, J. (2009). Are animal models predictive for humans? *Philosophy, Ethics, and Humanities in Medicine : PEHM*, *4*(2). https://doi.org/10.1186/1747-5341-4-2

Smith, J. S., Mochel, J. P., Soto-Gonzalez, W. M., Rahn, R. R., Fayne, B. N., Escher, O. G., Geletka, A. M., … & Cox, S. (2021). Pharmacokinetics of Pantoprazole and Pantoprazole Sulfone in Goats After Intravenous Administration: A Preliminary Report. *Frontiers in Veterinary Science*, *8*(September), 1–6. https://doi.org/10.3389/ fvets.2021.744813

Soares, G. A., Pires, D. W., Pinto, L. A., Rodrigues, G. S., Prospero, A. G., Biasotti, G. G. A., Bittencourt, G. N., … & Miranda, J. R. A. (2021). The influence of omeprazole on the dissolution processes of ph-dependent magnetic tablets assessed by pharmacomagnetography. *Pharmaceutics*, *13*(8). https://doi.org/10.3390/ pharmaceutics13081274

Solhaug, M. J., Bolger, P. M., & Jose, P. A. (2004). The Developing Kidney and Environmental Toxins. *Pediatrics*, *113*(4 II), 1084–1091.

Takashi, Kuramoto (Institute of Laboratory Animals, Graduate Scholol of Medicine, K. U. (2012). Origin of Albino Laboratory Rats. *Research and Bioresources*, *12*(8).

Thélin, C. S., & Richter, J. E. (2020). Review article: the management of heartburn during pregnancy and lactation. *Alimentary Pharmacology and Therapeutics*, *51*(4), 421–434. https://doi.org/10.1111/apt.15611

Tran, T., Sundaram, C. P., Bahler, C. D., Eble, J. N., Grignon, D. J., Francesca Monn, M., Simper, N. B., & Cheng, L. (2015). Correcting the shrinkage effects of formalin fixation and tissue processing for renal tumors: Toward standardization of pathological reporting of tumor size. *Journal of Cancer*, *6*(8), 759–766. https://doi.org/10.7150/jca.12094

Tytgat, G. N. (2001). Shortcomings of the first-generation proton pump inhibitors. *European Journal of Gastroenterology & Hepatology*, *13*(Suppl 1*)*, S29-33.

van der Pol, R. J., Smits, M. J., van Wijk, M. P., Omari, T. I., Tabbers, M. M., & Benninga, M. A. (2011). Efficacy of proton-pump inhibitors in children with gastroesophageal reflux disease: a systematic review. *Pediatrics*, *127*(5), 925–935. https://doi.org/10.1542/peds.2010-2719

Van Rensburg, C. J., & Cheer, S. (2012). Pantoprazole for the Treatment of Peptic Ulcer Bleeding and Prevention of Rebleeding. *Clinical Medicine Insights: Gastroenterology*, *5*, CGast.S9893. https://doi.org/10.4137/cgast.s9893

Vlajković, S., Daković-Bjelaković, M., Cukuranović, R., & Popović, J. (2005). Evaluation of absolute volume of human fetal kidney's cortex and medulla during gestation. *Vojnosanitetski Pregled. Military-Medical and Pharmaceutical Review*, *62*(2), 107–111. https://doi.org/10.2298/vsp0502107v

Walpole, S. C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., & Roberts, I. (2012). The weight of nations: An estimation of adult human biomass. *BMC Public Health*, *12*(1), 1. https://doi.org/10.1186/1471-2458-12-439

Xie, Y., Bowe, B., Li, T., Xian, H., Yan, Y., & Al-Aly, Z. (2017). Long-term kidney outcomes among users of proton pump inhibitors without intervening acute kidney injury. *Kidney International*, *91*(6), 1482–1494. https://doi.org/10.1016/j.kint.2016.12.021

Yepuri, G., Sukhovershin, R., Nazari-Shafti, T. Z., Petrascheck, M., Ghebre, Y. T., & Cooke, J. P. (2016). Proton Pump Inhibitors Accelerate Endothelial Senescence. *Circulation Research*, *118*(12), e36-42. https://doi.org/10.1161/CIRCRESAHA.116.308807

Yoshikawa, I., Nagato, M., Yamasaki, M., Kume, K., & Otsuki, M. (2009). Long-term treatment with proton pump inhibitor is associated with undesired weight gain. *World Journal of Gastroenterology*, *15*(38), 4794–4798. https://doi.org/10.3748/wjg.15.4794

Zhou, S., Xie, R., Zhang, X., He, X., Huang, J., Jungang, Y., Liao, M., … & Cui, Y. (2022). Evaluation of the relationship between polymorphisms in CYP2C19 and the single-dose pharmacokinetics of omeprazole in healthy Chinese volunteers: A multicenter study. *Clinical and Translational Science*, *15*(6), 1439–1448. https://doi.org/10.1111/cts.13255

Zielinski, R., Searing, K., & Deibel, M. (2015). Gastrointestinal distress in pregnancy: Prevalence, assessment, and treatment of 5 common minor discomforts. *Journal of Perinatal and Neonatal Nursing*, *29*(1), 23–31. https://doi.org/10.1097/JPN.0000000000000078

Zoetis, T., & Hurtt, M. E. (2003). Species Comparison of Anatomical and Functional Renal Development. *Birth Defects Research Part B - Developmental and Reproductive Toxicology*, *68*(2), 111–120. https://doi.org/10.1002/bdrb.10013

APPENDICES

Appendix I: Ethical approval

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2021/328

Ms. Ann Njoki Nyaga.
Dept. Human Anatomy,
JKUA & Technology.
11/11/2021

Dear Ann,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

Comparative morphological and histostereological teratogenic effects of in-utero exposure to varied doses of omeprazole and pantoprazole on fetal kidneys of albino rats.

Ann Njoki Nyaga. HSM301-1195/2020.

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 8th November 2021. We have reviewed your application for ethical clearance for the study. The number of rats, animal husbandry practices and the proposed protocol that will be used to compare morphological and histostereological teratogenic effects of *in-utero* exposure to Omeprazole and Pantoprazole on the fetal kidneys meets the minimum standard of the Faculty of Veterinary medicine, Biosafety and Animal use and Ethical regulation guidelines.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix II: Publication

Journal of Agriculture Science & Technology JAGST 22 (4) 2023, 53-63

ORIGINAL RESEARCH ARTICLE

The effects of prenatal exposure to varying doses of pantoprazole on the maternal and fetal outcomes in albino rats *(rattus norvegicus).*

***Anne Njoki Nyaga*[1], *Joseph Kariuki Kweri*[1], *James Mwangi Kanyoni*[1], *Ann Wairimu Mwangi*[1], *Caroline Chepng'eno Sigei*,[1] *Jennifer Chepkemoi Segut*[1], *Jane Wanjiru Kuria*[2]**

[1]*Department of Human Anatomy, School of Medicine (SOMED), College of Health Sciences (COHES), Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya.*
[2]*Department of Clinical Medicine, Kirinyaga University KYU, Kenya.*

Corresponding author email: nyaganne09@gmail.com

Abstract
Pantoprazole is a proton pump inhibitor used in the management of hypergastric secretions and gastroesophageal reflux during pregnancy. However, its prenatal effects on maternal and fetal outcomes are not well reported when administered at varying doses and at different gestational periods. A post-test-only experimental study design was adopted in conducting this study. A sample size of 30 female albino rats was used for the study. The 30 albino rats were grouped into two broad study categories: 3 control rats and 27 treatment rats. The 27 treatment rats were subdivided into three study groups of nine rats each according to the doses administered as follows: 9 low-dose rats, 9 medium-dose rats, and 9 high-dose rats The nine rats assemblies were further divided up into three subgroups, each of three rats, according to the time of exposure, as follows: three rats for trimester one, three rats for trimester two, and three rats for trimester three. Daily maternal weights were recorded every morning, and then at gestation day 20, all animals were humanely sacrificed and the fetuses harvested. Continuous data included the maternal and fetal weights, and discrete data included the litter sizes, number of devoured fetuses, resorbed glands, and number of embryolithalities. Data was recorded, coded, and entered in the computer using MS Excel spreadsheets version 13, and analyzed using the SPSS programme for Windows version 25 (one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc multiple comparisons test). The results were expressed as means ± standard error of the mean (SEM). Results with a $P < 0.05$ were considered significant in the study. This study observed that pantoprazole, at high doses, was associated with a decrease in the mean maternal weight gain, reduced litter sizes with increased numbers of resorbed endometrial glands, and devoured fetuses.

Key words: Pantoprazole, proton pump inhibitor, fetuses, *In* –utero effects.

1.0 Introduction
Gastroesophageal reflux (GER) is the most common health challenge experienced by expectant mothers in the early and last stages of their pregnancy, with a prevalence rate of about 80%. It however tends to increase in severity with every consecutive trimester (Ali et al., 2022). Mothers in developing countries are hence commonly given PPIs, in particular pantoprazole, in an attempt to seek relief. Consequently, pantoprazole is among the most widely used PPIs in

Appendix III: Data Capture Sheet For Pregnant Albino Rats

Albino Rat Identity……

Initialweight……Dose Calculation……

DATE	**WEIGHT IN GRAMS**	**OMEPRAZOLE DOSE (mg/bwt)**	**GENERAL CONDITION OF THE RAT**

Appendix IV: Data Capture Sheet For The Albino Fetuses

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Gross Appearance									
FETAL WT(G)									
Obvious congenital Abnormalities Of The Fetus									
Resorptions									
Kidneys									
Gross Appearance									
Obvious Congenital Anomalies Of Kidneys									
KIDNEY WT(G) Rt Kidney									
Left Kidney									
Total Kidney Volume (Right)									
Total Kidney Volume (Left Kidney)									