

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

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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 \pm 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

Kenya and elsewhere in the developing nations of Africa (Pasternak & Hviid, 2011; Th  lin & Richter, 2020). Pantoprazole, which is in the class of proton pump inhibitors, is a substitute for beminidazole. It is widely used as it is considered safe and has been found to be quite efficacious in managing gastro-esophageal reflux. In addition, it is widely accessible as an over-the-counter medicine, hence preferred by both expectant mothers and healthcare providers (Body & Christie, 2016; Jewell, 2007). During the management of GER, it works by decreasing gastric acid secretion through the irreversible inhibition of the proton pump (H⁺/K⁺-ATPase) of the parietal cells in the gastric pit (Cheer et al., 2003; Comoglu et al., 2008). It is also notable that PPIs are the most widely prescribed medications in primary healthcare facilities because of their fast-relieving nature and the widespread notion that they have few adverse effects (Pasternak & Hviid, 2011). However, some recent studies have shown that PPI usage in pregnancy is linked with a number of deleterious effects, like inducing oxidative stress in the placenta and liver and also causing incomplete ossification of the fetal bones, chronic toxicity, and inducing gastric tumors as well as tumors in the liver (Alaa et al., 2019). The use of pantoprazole in pregnancy may thus pose a risk to the fetus, although the available data has not well shown how it interferes with the maternal prenatal environment where the fetus develops. This study is thus set to evaluate the *in-utero* effects of pantoprazole on maternal pregnancy outcomes, including maternal weight gain, maternal terminal weight, litter sizes, resorbed endometrial glands, devoured fetuses, and embryolithalities, based on varied doses of pantoprazole and when administered at different gestational periods in pregnant albino rats.

2.0 Materials and Methods

2.1 The Study setting

The animal experimentation processes that included mating and confirmation of pregnancy, feeding, taking of daily weights, general observation of the rats, daily drug administration, humane sacrificing, tissue harvesting, and measurements of maternal and fetal parameters were carried out in the Animal house located in the School of Biological Sciences at the University of Nairobi, Chiromo campus.

2.2 Study design

In carrying out this study, a post test only control experimental study design was adopted

2.3 Study subjects

A total of 30 female albino rats obtained from a pure colony bred from a 4th generation aged between 7 and 8 weeks old and weighing 190 ± 30 grammes were used as the experimental model. The albino rats were preferred as the experimental model since they are proven to have the following well-known scientific facts: (i) have a bigger litter size ranging between 5 and 16 fetuses; (ii) the chances of spontaneous congenital defects are low; (iii) fairly short gestational span; (iv) the cost of maintenance is less; (v) are plentiful; (vi) the availability of data on reproduction; (vii) handling and caring for them is easier since they are small in size; (viii) they can withstand a wide range of medicines used in the animal studies (Bailey et al., 2014; Pritchett & Corning 2016). The rats were kept in spacious polycarbonate plastic cages that had bar lids to hold the feed and water bottles (Kanyoni J Mwangi *et al.*, 2023) An ambient environmental

condition of optimal temperature, relative humidity, and a 12-hour light/dark cycle was maintained for the entire experimental period.

2.4 Sample size determination

The resource equation of group comparison for one-way Analysis of Variance was adopted in determining the number of rat dams that were to be used in the study. This equation was adopted because it can be applied to all animal experimental studies (Charan & Kantharia, 2013) and also because, based on the existing literature from previous studies, the standard deviation and the effect on the sample size are not available (Arifin and Zahiruddin, 2017). In the method, the value 'E' was measured and used as the degree of freedom in the analysis of variance (ANOVA) based on the determined sample size. The formula is $n = DF/k + 1$, where DF is the total number of subjects, k is the number of groups, and n is the number of subjects per group (Charan & Biswas, 2013). Therefore, $n = 20/10+1 = 3$. The sample size of the fetuses was determined using the convenient random sampling method, where from each pregnant woman in the study, three fetuses were selected; thus, the total number of fetuses for analysis was 3x30, or 90 fetuses.

2.5 Mating and confirmation of pregnancy in the rats

Mating was done after one week of acclimatization by introducing one sexually mature male albino rat (7–8 weeks) obtained from the same pure-bred colony of the fourth generation to a standard plastic cage where two female albino rat dams were housed. Mating was allowed to take place overnight (1200 hrs). The males were then put into their separate cages the following day. Mating was confirmed by obtaining vaginal smears from the mated females and examining them for the presence of spermatozoa. Pregnancy was confirmed by the presence of polyhedral epithelial cells on the vaginal swabs microscopically.

2.6 Grouping of the 30 experimental rats

Following the confirmation of pregnancy, the thirty (30) rats were first grouped into two broad study categories: Three (3) rats as the control and twenty seven (27) rats as the treatment group. To find out whether the effects of pantoprazole were dose-dependent, the twenty-seven (27) dams in each treatment group were further split into three study groups of nine (9) dams each in line with the dosages administered: 9 dams for the low dose (4.13 mg/kg BW) pantoprazole group; 9 dams in the medium dose (13.43mg/kg BW) pantoprazole group; and lastly, 9 dams in the high dose (24.8 mg/kg BW) pantoprazole category. To ascertain if the effects of pantoprazole were dependent on time of exposure, the nine dams in each of the three already established study groups of low, medium, and high dose pantoprazole were further subdivided into three subgroups, each composed of three rats, according to the period of exposure. Thus, three (3) rats were placed in trimester one (TRM1), three (3) rats in trimester two (TRM2), and three (3) rats in trimester three (TRM3).

2.7 Weighing and feeding of the rats

Weighing of the control and treatment animals was done every morning using a precision weigh scale, and weight was recorded down. Additionally, the animal feeding was done every morning at 9:00 a.m. for both the control group and experimental group rats using standard rodent

pellets and water *ad libitum* (Kanyoni James Mwangi *et al.*, 2023) for the entire gestation period from day 1 to the 20th day.

2.8 The acquisition of Pantoprazole and calculation of human to rats' doses

Pantoprazole sodium 40mg (batch number PAU20001ES) used in the study. The medicine was soluble in distilled water. The dosages were calculated using the guide for dose conversion between humans and animals (Nair *et al.*, 2018), which bases the dosage on the animal's weight and body surface area as follows: Human Equivalent Dose (HED): mg/kg = Animal dose in mg/kg multiplied by a constant ratio (Km) of 6.2. The minimum adult dose for pantoprazole is 40mg/day, the medium dose is 130 mg/day, and the maximum dose for humans is 240mg/day. The human average weight (60kg) was used to calculate the human equivalent dose. The minimum, medium, and maximum dosages for humans were divided by 60 to get human equivalent doses in Mg/Kg. This was further multiplied by a constant Km factor of 6.2 to get the animal equivalent doses (AED) in mg/kg as follows: low dose 4.1333mg/Kg, medium dose 13.4354 mg/kg, and high dose 24.8mg/kg. This was further divided by 1000 grams so as to get doses in milligrams. To finally get the actual doses administered to the rats, the real weight of each rat was multiplied by the AED.

2.9 Administration of Pantoprazole

The experimental rats received pantoprazole treatment, which was administered using a gavage needle gauge 16(Ann *et al.*, 2023) according to the calculated dosages of low, medium, and high doses and in accordance with the period of exposure as trimester one, trimester two, and trimester three. Those in trimester one received pantoprazole from day 1 of the gestation to the 20th gestation day. The rats in trimester two were introduced to pantoprazole from gestation day 7 up to the 20th day, while rats in trimester three received pantoprazole from gestation day 14 up to the 20th day.

2.10 Humanely sacrificing of the rats and tissue handling

All rat dams in both the control and treatment groups were monitored throughout the pregnancy and humanely sacrificed after euthanizing the animal using concentrated carbon dioxide on the 20th gestation day. Tissue harvesting and measurement of maternal and fetal parameters were done and recorded on the structured data capture sheets.

2.11 Statistical analysis

Data analysis was performed using the One-way Analysis of Variance (ANOVA). P values of less than 0.05 ($P < 0.05$) were considered to be statistically significant.

3.0 Results

3.1 The effects of pantoprazole on daily mean maternal weight trends during gestation period.

It was observed that the mean maternal daily weight trends for the animals in the control group increased steadily all through the gestation period. Further, in the treatment groups, the rats that received low and medium dosages of pantoprazole from trimester one showed an upward trend in their mean daily maternal weight. However, in the high-dose group, it was observed that there was weight stagnation for the first three days following the introduction of

pantoprazole, followed by an upward weight trend, even though the daily mean weight trend was low when compared to the control group (figure 1).

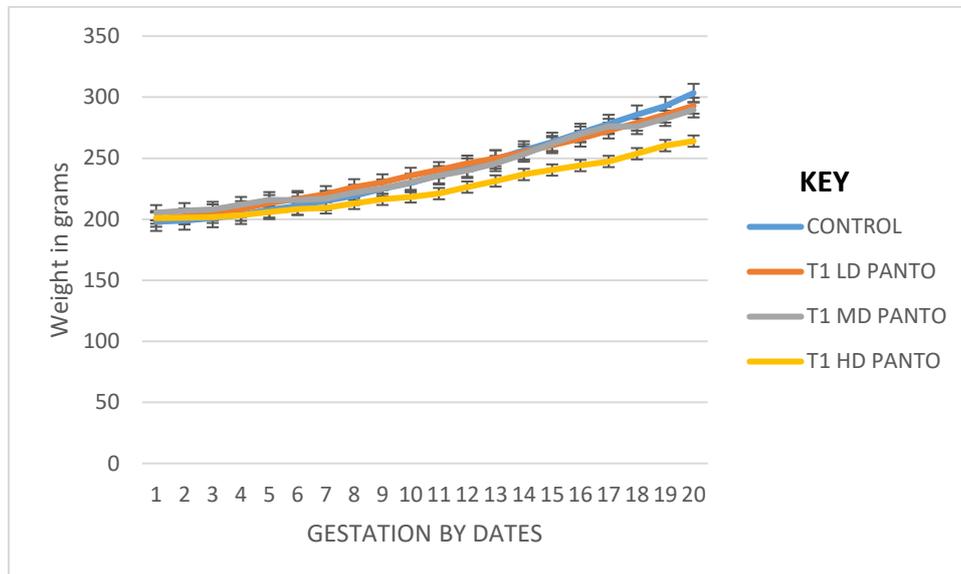


Figure 1 shows daily mean weight trends for low, medium, and high-dose pantoprazole in trimester one.

Following the introduction of pantoprazole in trimester two, it was observed that the daily mean maternal weight for the rats in the low-dose group had an upward trend throughout the gestation period. The daily mean maternal weight for the medium-dose rats' category was also on an upward trend, although the trend was slightly negatively affected following the introduction of medium-dose pantoprazole. For the high-dose pantoprazole group, the mean daily maternal weight dropped after the drug was introduced but thereafter maintained an upward trend. The control group animals had an upward daily mean maternal weight throughout the gestation period (Figure 2).

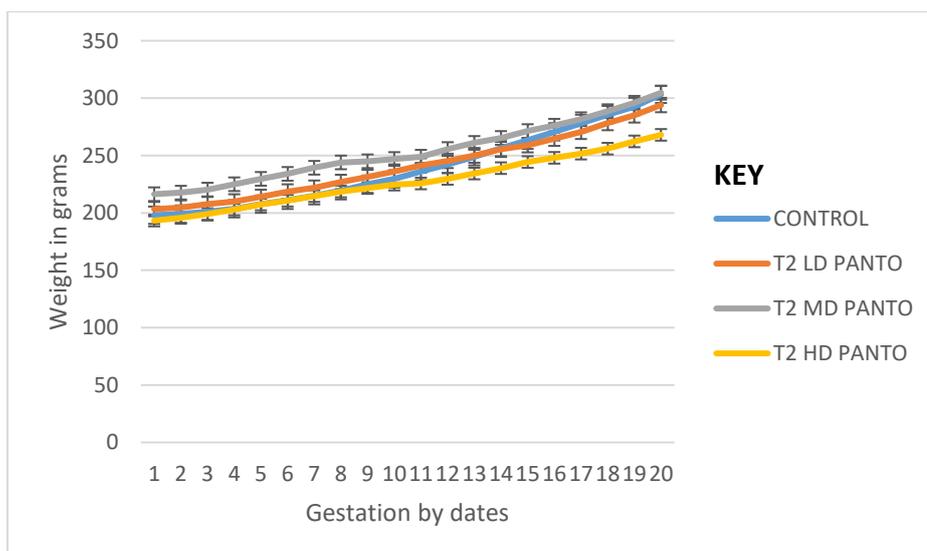


Figure 2 shows daily mean weight trends for low, medium, and high pantoprazole in trimester two.

The daily mean weight trend for the rats in the third trimester showed an upward trend in both the treatment and control groups. However, the rats had a drop in the daily mean weight when high-dose pantoprazole was administered during the third trimester (Figure 3).

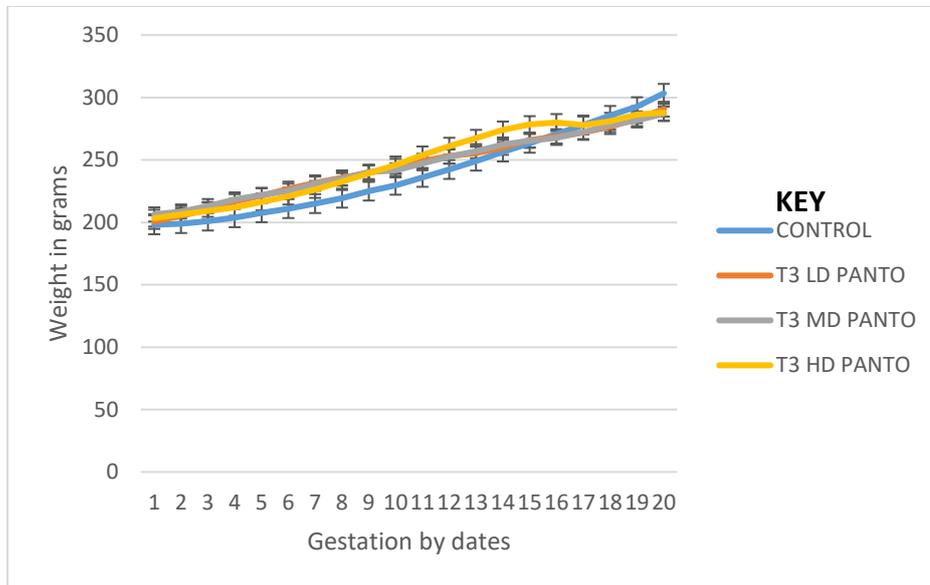


Figure3. Shows daily mean weight trends for low, medium, and high pantoprazole in trimester three.

3.2 The perinatal effects of pantoprazole on maternal weight gain.

It was observed that mean weight gain in the low-dose and medium-dose treatment categories did not differ statistically when this was compared with the mean weight gain of the control group ($P > 0.05$). However, the mean weight gain in the high dose categories across the three trimesters was significantly lower following the administration of pantoprazole as compared to the control.

Table 1. TRM1, TRM2, and TRM3 initial weight, terminal weight, and weight gain for low, medium, and high doses against control.

Study group	Treatment period	Initial weight	Terminal weight	Weight gain (SEM)
Control		195.50±2.500	303.33±4.096	105.33±4.096
Low dose	TRM 1	202.67±7.219	299.00±4.041	96.33 ± 3.480
	TRM 2	205.3333±4.6667	286.33±6.386	81.00 ± 7.506
	TRM 3	201.0000±4.6188	292.67±2.082	54.67 ± 8.253
Medium dose	TRM 1	203.3333±2.6034	286.00±3.512	86.67 ± 1.764
	TRM 2	214.0000±2.646	290.67±2.333	76.67 ±1.202
	TRM 3	197.0000±2.517	290.33±3.464	93.33 ±3.667
High dose	TRM 1	203.3333±0.3333	255.67±4.702*	65.67±1.856*
	TRM 2	193.3333±3.9299	253.33±0.882*	84.00±4.359*
	TRM 3	201.6667±5.8119	269.67±2.333*	92.00±2.887*

Key TRM 1 =Trimester 1, TRM 2=Trimester 2, TRM 3 =Trimester 3

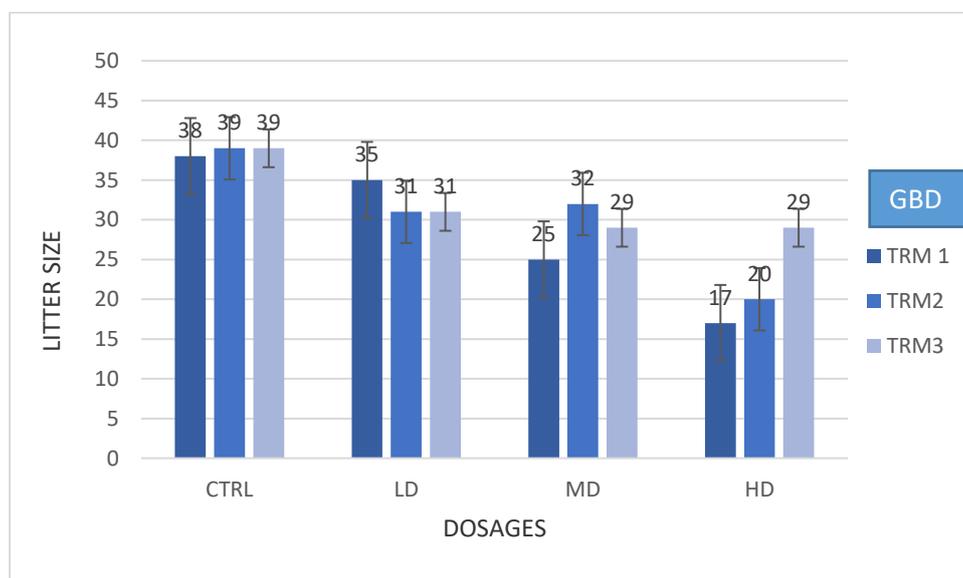
(*) signifies a statistical significance at $p < 0.05$ when compared to control.

3.3 The perinatal effects of pantoprazole on mean maternal terminal weights

All rats that received the low and medium doses of pantoprazole across the three trimesters had terminal weights that did not differ statistically ($P > 0.05$) when compared with the mean terminal weight of the control group. In contrast, the mean terminal weight differed across the trimesters when high doses were administered compared with the control group. For instance, the mean terminal weight of the control group differed statistically with that of high-dose animals that had been subjected to pantoprazole in trimester one (TM1) at $P = 0.001$.

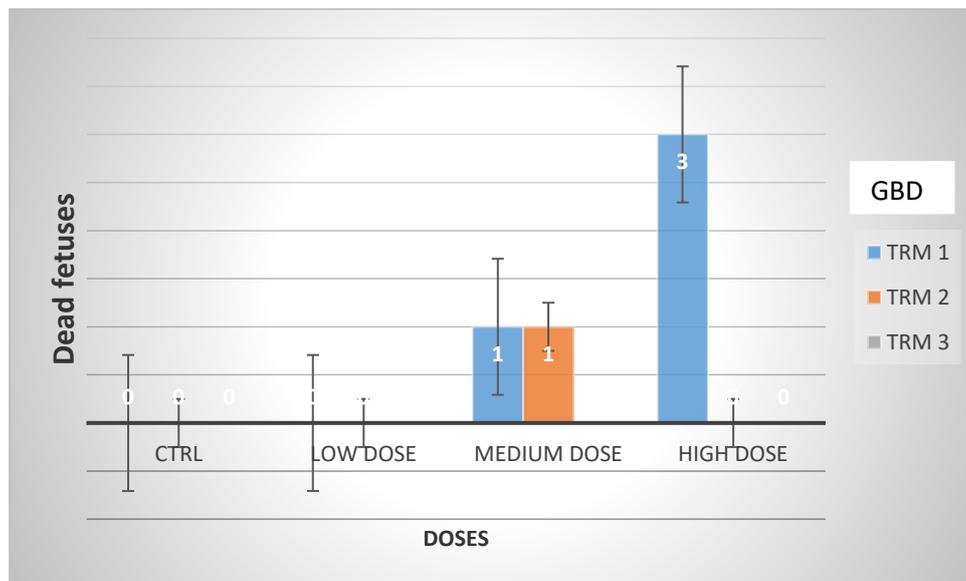
3.4 The effects of perinatal exposure to pantoprazole on the litter size and *in-utero* fetal deaths (embryolithalities)

The litter size was highest in the control group category, followed by the rats that received low-dose pantoprazole, then the medium-dose category, and lowest in the high-dose category (graph 1).



Graph 4. Shows the comparison of the mean litter size of the treatment groups against the control group

It was observed that there was no fetal death in the control group and low-dose group categories, as opposed to trimester one and two medium-dose groups, which had one fetal death each. The high-dose pantoprazole group had the highest number of fetal deaths (graph 5).



Graph 5. Shows the distribution of dead fetuses in the treatment groups against the control

3.5 Resorbed glands

The number of resorbed glands was highest in the rats that received high-dose pantoprazole during the first trimester (TRM₁HD₁) (12), followed by those that received medium-dose pantoprazole (TRM₁MD₂) 5, and TRM₂HD₂) (4). It was, however, noted that there were no resorbed glands in the control group or the rats that received low-dose pantoprazole.

4.0 Discussion

The findings of this study include the daily mean maternal weight, the mean terminal weight, the mean weight gain, the litter size, the number of dead fetuses, and the number of resorbed endometrial glands.

4.1 The maternal pregnancy outcomes on daily mean weight, mean maternal terminal weight, and mean maternal weight gain.

According to the results of this study, it was observed that animals in the control group and those that received low-dose pantoprazole didn't differ statistically in their mean terminal weight (table 2). The current results are in agreement with a previous study done by Aykan & Ergun (2018), whereby the study results showed that the terminal weight for both the control and low dose categories didn't vary. However, there was a significant change in the terminal weight for the high-dose group irrespective of the trimester, although this was observed more in trimesters one and two when contrasted with the control group. This current study's results are found to be consistent with a prior study by Alaa *et al.* (2019) who observed that the mean terminal weight was affected in a dose-dependent manner upon administration of drugs in the same classification as pantoprazole. This could be relate to the observation that there was reduced placental weights and drug-induced oxidative stress in pregnant rats when the drug is used for a prolonged period of time.

In the current study, the control group category was observed to have the highest mean maternal weight gain, while the least mean weight gain was observed in the rats receiving the high dose of pantoprazole. This was more marked during the first trimester (TRM₁), followed by trimester two (TRM₂), and lastly trimester three (TRM₃) (table 2). These results on mean maternal weight gain, however, contradict those of a previous study by Shirazi *et al.* (2014), which indicated there was no major weight gain or loss when pantoprazole was used.

4.2 The effects of prenatal exposure to pantoprazole on litter size, fetal death, and fetal resorption

The control group was not associated with any fetal deaths in this study. However, they were observed in the medium and high dosage categories (graph 5). Similarly, resorbed glands were noted in animals in the high and medium dose categories. The current results concur with those of previous studies done on small experimental animals where certain PPIs like omeprazole, a drug in the same class as pantoprazole, were associated with disruption of pregnancy, fetal resorption, and resultant damage to the embryo, especially when administered at high doses throughout the pregnancy (Alaa *et al.*, 2019; Mathews *et al.*, 2010).

Further, these study results demonstrated that the mean litter size was highest in the control group, followed by the low dose group, then the medium dose group, and lastly, the high dose group (graph 4). This direct dose-related reduction in litter size agrees with a previous study whose results on pregnant rats also showed a reduction in litter sizes when high doses of omeprazole were administered. (EMEA, 2002). There was no maternal death in the treatment rats or miscarriage that was recorded during the study.

5.0 Conclusion and recommendations:

Following this study, the conclusion is drawn that the use of pantoprazole at low dosages is not teratogenic. However, when applied at medium and high doses equivalent to therapeutic doses used in humans, it is associated with a risk to the pregnancy, especially when used from trimester one during organogenesis. Further study is recommended to be done with non-human primates that have close associations with humans to determine the most appropriate doses that are safe for humans, but for now, pantoprazole at high doses should be avoided, particularly during pregnancy.

6.0 Acknowledgement

6.1 General acknowledgment

None

6.2 Funding

None

6.3 Conflict of interest

The author declares no conflict of interest.

6.4 Ethical consideration



All the experimental procedures were performed as per the established protocols and regulations for the care and handling of animals as prescribed by the International Animal Research Institute (IARI) and the guidelines for the care of laboratory animals with approval from the Animal Ethics and Research Committee of the University of Nairobi (REF: FVM BAUEC/2021/328).

7.0 References

- 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.
- 13 Pantoprazole effects on pregnant rats.pdf
- Ali, R. A. R., Hassan, J., & Egan, L. J. (2022). 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-w>
- Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. *Malays JMed Sci*. 2017;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>
- 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>
- Charan, J., & Kantharia, N. (2013). How to calculate sample size in animal studies? *Journal of Pharmacology and Pharmacotherapeutics*, 4(4), 303–306. <https://doi.org/10.4103/0976-500X.119726>
- 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>
- Comoglu, T., Gonul, N., Dogan, A., & Basci, N. (2008). Development and in vitro evaluation of pantoprazole-loaded microspheres. *Drug Delivery*, 15(5), 295–302. <https://doi.org/10.1080/10717540802006864>
- EMA. (2002). Committee for Veterinary Medicinal Products. OMEPRAZOLE. SUMMARY REPORT. The European Agency for the Evaluation of Medicinal Products Veterinary Medicines and Inspections, June, 6. <http://www.emea.eu.int>
- Jewell, R. (2007). Pantoprazole. *XPharm: The Comprehensive Pharmacology Reference*, 1–5. <https://doi.org/10.1016/B978-008055232-3.62361-X>
- Mwangi, K. J., Kariuki, K. J., Elijah, M., Dominic, M., Wairimu, M. A., & Walter, R. (2023). The ameliorative effects of graded intensities of exercise training on anthropometrical parameters on high fat diet and sucrose-induced obesity in Wistar rats. *Journal of Agriculture, Science and Technology*, 22(1), 26-36.



- Mwangi, K. J., Kariuki, K. J., Githinji, M. E., Muriithi, K. A., Dominic, M., Wairimu, M. A., & Walter, R. K. (2023). The restorative effects of graded intensities of exercise training on the biochemical and nutritional status of obese induced male Wistar rats (*Rattus novogicus*). *Journal of Agriculture, Science and Technology*, 22(3), 39-50.
- Mwangi, A. W., Kweri, J. K., Kweri, C. K., Kanyoni, J. M., Kigundu, A. M., Mwangi, E. G., & Marera, D. O. (2023). The histostereological teratogenic effects of in-utero exposure to varied doses of lamotrigine on the developing fetal brain in albino rats (*Rattus Norvegicus*). *Journal of Agriculture, Science and Technology*, 22(3), 51-63.
- 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>
- 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>
- 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>
- Shirazi, M., Alimoradi, H., Kheirandish, Y., Etemad-Moghadam, S., Alaeddini, M., Meysamie, A., Fatahi Meybodi, S. A. R., & Dehpour, A. R. (2014). Pantoprazole, a proton pump inhibitor, increases orthodontic tooth movement in rats. *Iranian Journal of Basic Medical Sciences*, 17(6), 448–453. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4137949/>
- 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>