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The Effects Of Prenatal Exposure To Varying Doses Of Pantoprazole On The Maternal-Pregnancy Outcomes In Albino Rats *(Rattus Norvegicus)*.

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ABSTRACT: *Pantoprazole is a proton pump inhibitor used in the management of hyper gastric secretions and gastroesophageal reflux during pregnancy. However, its prenatal effects on maternal pregnancy 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 of 3 control rats and 27 experimental rats. The 27 experimental 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 9 rats assemblies were further divided into three subgroups; of 3 rats each according to the time of exposure as follows; 3 rats for trimester one, 3 rats for trimester two, and 3 rats for trimester three. Daily maternal weights were recorded every morning, 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, Ms Windows Excel spreadsheets version 13, and analyzed using the SPSS program 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 whose 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.*

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I. Introduction

Gastroesophageal reflux (GER) is the most common health challenge experienced by expectant mothers in the early and the last stages of their pregnancy with a prevalence rate of about 80% (Dong Seok et al., 2021). It however tends to increase in severity with every consecutive trimester(Ali *et al.,* 2022). Mothers in developing countries are hence commonly given PPIs and in particular pantoprazole in an attempt to seek relief. Consequently, pantoprazole is among the widely used PPIs in Kenya and elsewhere in the developing nations of Africa (Pasternak & Hviid, 2011; Thélin & Richter, 2020). Pantoprazole which is in the class of proton pump inhibitor is a substitute of beminidazole. It is widely used as it is considered safe and has been found to be quite efficacious in managing gastro-esophageal refluxes. In addition, it is widely accessible as an over-the-counter medicine and hence preferred by both expectant mothers and healthcare givers (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 health care facilities because of their fast relieve nature and a widespread notion that they have few adverse effects(Pasternak & Hviid, 2011). However, some recent studies have shown that PPIs usage in pregnancy is linked with a number of deleterious effects like inductive oxidative stress to the placenta and liver and also causing incomplete ossification of the fetal bones, chronic toxicity, inducing gastric tumors as well as tumors in the liver (Alaa *et al*., 2019; GlaxoSmithKline Inc., 2016). The use of pantoprazole in pregnancy may thus pose a risk to fetus although the available data has not well shown how it interferes with maternal prenatal environment

where the fetuses develops. This study thus set to evaluate the *in-utero* effects of the pantoprazole on the maternal pregnancy outcomes including maternal weight gain, maternal terminal weight, litter sizes, resorbed endomentrial glands, devoured fetuses and embryolithalities based on varied doses of pantoprazole and when administered at different gestational periods in pregnant albino rats.

II. Materials and methods

The Study setting: The animal experimentation processes that included mating and confirmation of the 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 in the University of Nairobi, Chiromo campus.

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

Study subject: A total of 30 female albino rats obtained from a pure colony rats bred from a 4^{th} generation aged between 7 to 8 weeks old and weighing 190±30grams, 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 5-16 fetuses, (ii) Chances of spontaneous congenital defects is low iii) fairly short gestational span, (iv) the cost of maintenance is less, (v) are plentiful (vi) availability of data on reproduction vi) handling and caring for them is easier since they are small in size (vii) 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 feeds and water bottles (Allen et al., 2016). An ambient environmental condition of optimal temperature, relative humidity and 12 hour light/dark cycle was maintained for the entire experimental period.

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, based on the existing literature from previous studies, where the standard deviation and the effect on the sample size is not available (Arifin & Zahiruddin, 2017). In the method, the value 'E' was measured and was used as the degree of freedom in analysis of variance (ANOVA) based on determined sample size. The formula is n = DF/k + 1, where DF = total number of subjects, k = number of groups, and n = 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 dams in the study, three fetuses were selected, thus, the total number of fetuses for analysis was 3x30= 90 fetuses.

Mating and confirmation of pregnancy in the rats: Mating was done after a one week of acclimatization by introducing one sexually mature male albino rat (7 to 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 (1200hrs). 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. Preganacy was confirmed by the presence of polyhedral epithelial cells on the vaginal swabs microscopically.

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

Weighing and feeding of the rats: Weighing of the control and the experimental animals was done every morning using a precision weigh sweree and weight was recorded down. Additionally, the animal feeding was done e9:00 amorning at 9:00am for both the control group and experimental group rats using the standard rodent pellets and water *ad libitum* for the entire gestation period from day 1 to 20^{th} day.

The acquisition of Pantoprazole and calculation of human to rats' doses: Pantoprazole sodium 40mg, (batch number PAU20001ES) was obtained from a government chemist in Nairobi. The medicine was soluble in distilled water. The dosages were calculated using the guide for dose conversion between humans to animals (Nair *et al.*, 2018) which bases the dosage on the animal weight and body surface area as follows, Human Equivalent dose (HED) mg / kg = Animal dose in mg/kg multiplied by a constant ratio (Km) 6.2. The minimum adult dose for pantoprazole is 40mg/day, the medium dose is 130mg/day and a maximum dose of human 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 13.4354mg/kg and high dose 24.8mg/kg. This was further divided by 1000grams 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.

Administration of Pantoprazole: The experimental rats received pantoprazole treatment which was administered using a gavage needle gauge 16 according to the calculated dosages of low, medium and high doses and in accordance to the period of exposure as trimester one, trimester two and trimester three. Those in trimester one received the pantoprazole from day 1 of the gestation to the 20^{th} gestation day. The rats in trimester two were introduced to pantoprazole as from gestation day 7^{th} upto to the 20^{th} day while rats in trimester three received the pantoprazole as from gestation day 14 upto the 20^{th} day.

Humanely sacrificing of the rats and tissue handling: All rat dams in both the control and treatment group were monitored throughout the pregnancy and humanely sacrificed after euthanizing the animal using concentrated carbon dioxide on the 20^{th} gestation day. Tissue harvesting and measurement of maternal and fetal parameters were done, recorded on the structured data capture sheets.

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

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.

III. RESULTS

The effects of pantoprazole on daily mean maternal weight trends during the 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 experimental groups, the rats that receieved low and medium dosages of pantoprazole from trimester one depicted 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 daily mean weight trend was low when compared to the control group (figure1).

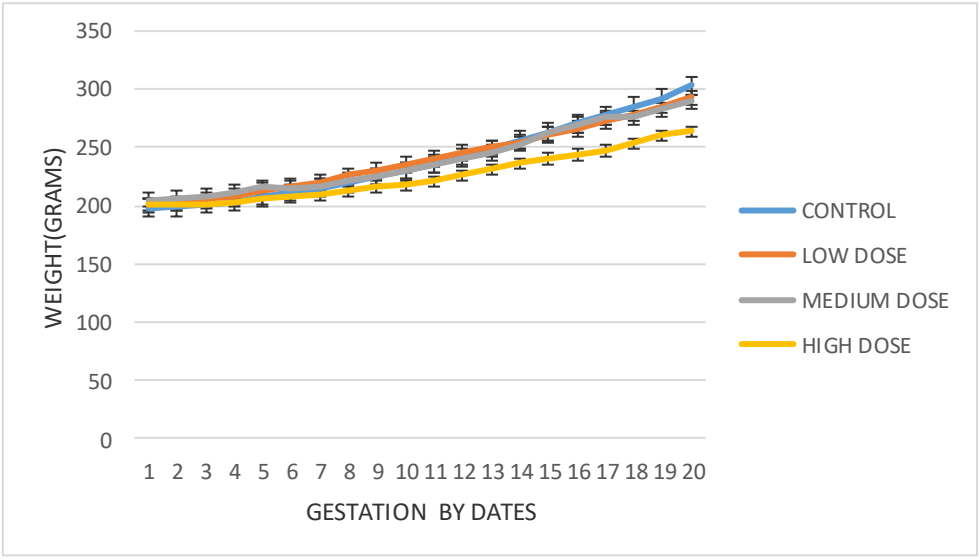


Figure 1 Showingaily mean weight trends for low, medium and high dose pantoprazole in trimester one.

Following the introduction of the 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 through out the gestation period. The daily mean maternal weight for the medium dose rats category was also on upward trend although trend was negatively affected slightly following the introduction of the medium dose pantoprazole. For the high dose pantoprazole group, the mean daily maternal weight had a drop after the pantoprazole 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).

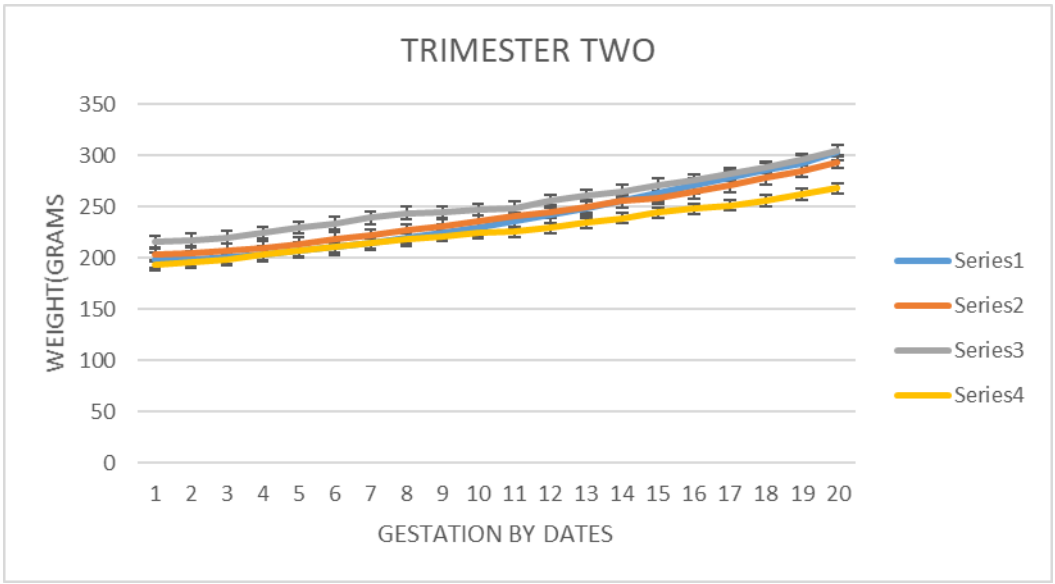


figure 2 showing daily mean weight trends for low, medium and high panroprazole in trimester two.

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

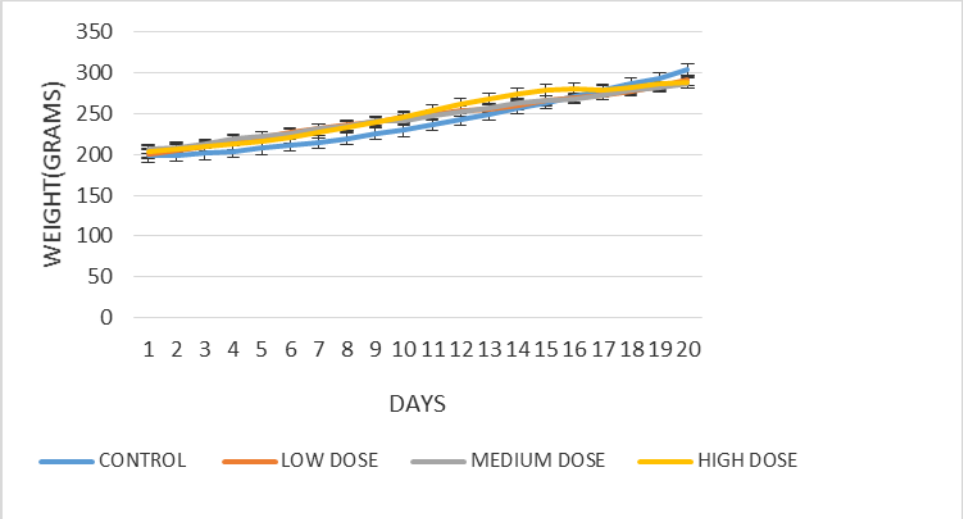


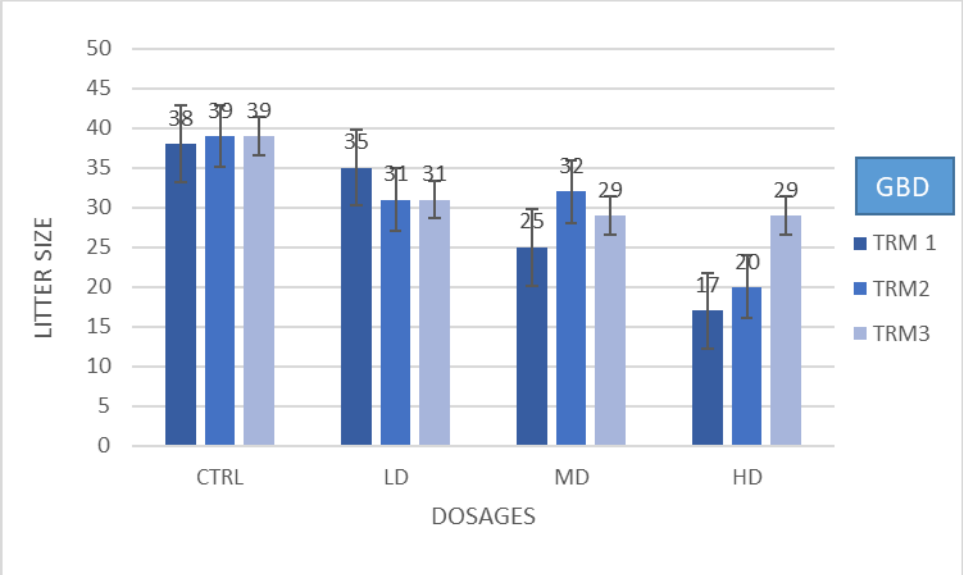
Figure3. Showing daily mean weight trends for low, medium and high pantoprazole in trimester three.

The perinatal effects of pantoprazole on the maternal weight gain.

It was observed that mean weight gain in the low dose and medium dose animal 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 low following the administration of pantoprazole as compared to the control.

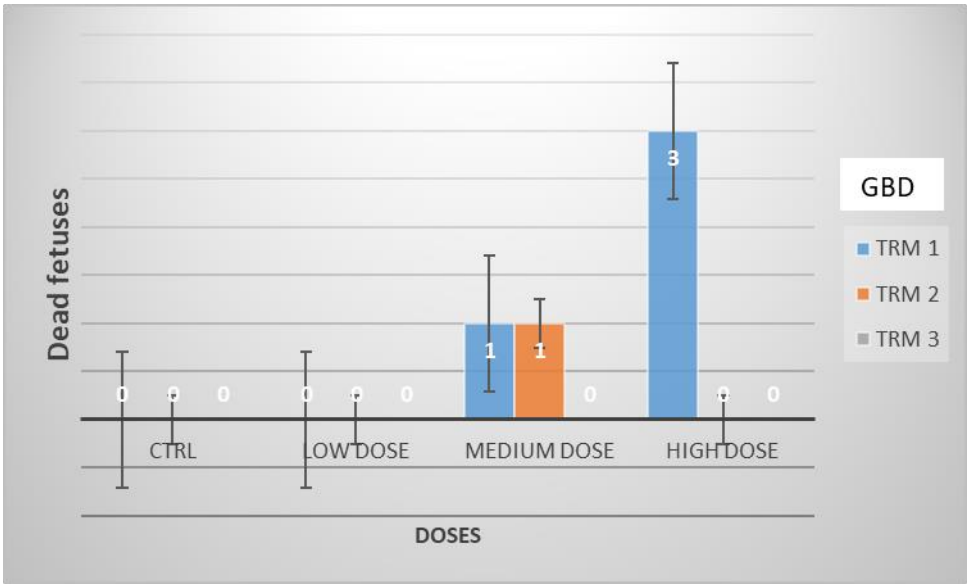
Table no.1. Showing TRM1, TRM2 and TRM3 initial weights, terminal weight and weight gain for low, medium and high dose against control.

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*



Graph 1. Showing 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 category as opposed to trimester one and two medium dose groups which had one fetal death each. High dose pantoprazole group had the highest number of fetal death (graph 5).



Graph 2. showing the distribution of the dead fetuses on the treatment groups against the control

Resorbed glands: The number of resorbed glands were highest in the rats that received high dose pantoprazole in during the first trimester ($TRM_1HD_{1)}$ (12) followed by those that received medium dose pantoprazole (TRM_1MD2) 5, ($TRM_2HD_{2)}$ had 4. It was however noted that there were no resorbed glands in the control group and the rats that received low dose pantoprazole.

IV. Discussion

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

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 1). 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 control and low dose category didn't vary. However, there was significant change in the terminal weight for the high dose group irrespective of the trimester, although this was observed more in the trimester one and two, when contrasted with the control group. This current study results is 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 explained by the fact that his results also found that there was reduced placental weights and the drug induced oxidative stress to the pregnant rats.

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 pantoprazole more marked during the first trimester(TRM_1) followed by trimester two(TRM_2) and lastly trimester three(TRM_3) (table 1). These results on mean maternal weight gain concurs with those of GlaxoSmithKline Inc., (2016), who reported that upon administration of high dose proton pump inhibitors(PPIs) in the same class with pantoprazole may have resulted in maternal drug toxicity hence low weight gain. This current study results however, contradicts those of a previous study by Shirazi *et al*.,(2014), that indicated there was no major weight gain or loss when pantoprazole was used.

The effects of prenatal exposure to pantoprazole on Litter size, fetal death and fetal resorption.

In this study, the control group was not associated with any fetal death. However, they were observed in the medium and high dosage categories (graph 2). Similarly, resorbed glands were noted in animals in the high and medium dose category. The current results concurs with those of previous studies done on small experimental animals where certain PPIs like omeprazole, a drug in the same class as pantoprazole was 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, this study results demonstrated that the mean litter size was highest in the control group followed by low dose group, then medium dose and lastly in the high dose group (graph 1). This direct dose related reduction in the number of litter size agrees with a previous study whose results on pregnant rats also showed a reduction in the litter sizes when high doses of omeprazole were administered. (EMEA, 2002). Generally, there was no maternal death of the experimental rats nor miscarriage that was recorded during the study.

V. Conclusion

Following this study, conclusion is drawn that use of pantoprazole at low dosages is not teratogenic. However, when applied at medium and high doses equivalent to therapeutic doses used in the human is associated with risk to the pregnancy especially when used as from trimester one during organogenesis.

VI. Recommendation

Further study is recommended to be done with non human primates that have close association to human to determine the most appropriate doses that are safe to humans but for now pantoprazole at high doses should be avoided particulary during the pregnancy.

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Declaration of interest

The author declares no conflict of interest.

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