Research Article

Teratogenic Effects of Pantoprazole on the Pregnant Rats and Their Fetuses during Gestation

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ABSTRACT

This study was undertaken to evaluate the teratogenic effects of the proton pump inhibitor (PPI) drug (pantoprazole) which was on a daily basis orally-administered to the pregnant albino rats (Rattus norvegicus). The virgin female rats were mated with the male and the pregnant rats were orally-administered a human equivalent dose (4.11 mg/kg) of Pantoprazole from 6th to 19th gestation days. On the 19th day of gestation, females were sacrificed and fetuses were removed from the uterus and evaluated for mortality rate, growth parameters, morphological and skeletal malformation and the oxidative stress markers were evaluated. Results showed decreased in corrected mother weight gain of pregnant rats and fetal growth retardation. Hematomas were detected morphologically in the fetuses and high incidence of resorption in treated animals. Fetal skeletal anomalies summarized as less degree of ossification in most bones, costal separation, curved and wavy ribs. Biochemical studies in both pregnant rats and their fetuses showed that pantoprazole induced a reduction in the level of reduced glutathione (GSH) which is an important intracellular (non-enzymatic antioxidant), and depletion in the superoxide dismutase (SOD) (enzymatic antioxidant) activity compared to control group. Also, elevation in the level of catalase (CAT) and Lipid peroxide Malondialdehyde (MDA) (Biomarkers of oxidative stress) compared to control group. All of these prove the teratogenic effect of the pantoprazole. Thus, we suggest the need for a great caution to handle pantoprazole especially during pregnancy.

Keywords: Pregnant albino rats, Skeletal abnormalities, Gastroesophageal reflux disease (GERD), Liver, Placenta, Pantoprazole and Oxidative stress.

INTRODUCTION

The birth defects could be obvious or latent at birth, owing to the conjugation effects of internal and external factors during the prenatal developmental processes. [1,2] Teratogenic agents like chemicals (drugs), irradiation and contagious substances. Potentially almost any medication used by the mother during pregnancy could be deleterious to the fetus, causing an anatomic defect (teratogenic).

Most pregnant women have symptoms of gastroesophageal reflux disease (GERD), a condition that develops when the reflux of stomach contents causes troublesome symptoms and/or complications**,** especially heartburn at some point. These symptoms may start at any time during a pregnancy and they often get worse throughout the pregnancy.[3, 4] General management plan would include the following: dietary and lifestyle modification as avoidance of tobacco, alcohol, chocolate, and citrus juice [5] and/or medical treatment if heartburn is not controlled by lifestyle measures as Antacids, Histamine H2-receptor Antagonists, Prokinetic and Proton Pump Inhibitors PPIs.

Proton Pump Inhibitors PPIs provide faster relief than prokinetics or H2-blocking agents and have good evidence for long-term healing of esophageal erosion (including Barrett's esophagus). Pantoprazole belongs to a class of drugs known as proton pump inhibitors (PPIs). It is used to treat certain stomach and esophagus problems (such as acid reflux). It helps heal acid damage to the stomach and esophagus; helps prevent ulcers, and may help prevent cancer of the esophagus. Pantoprazole is rapidly and completely absorbed after oral administration. It is almost exclusively metabolized in the liver through the cytochrome P450 (CYP) system. The main metabolic pathway is demethylation, by CYP2C19, with subsequent sulfation; other metabolic pathways include oxidation by CYP3A4. [6]

FDA Pharmaceutical Pregnancy Category (B) for pantoprazole in animal reproduction studies is not available for risk to the fetus classification. But there are no enough clinical trials or studies are available regarding the safety of Pantoprazole use during pregnancy. [7] The present study is carried out to evaluate the teratogenic potential of the proton pump inhibitor drug (Pantoprazole) on the female rats orally administrated with (4.11mg/kg) from 6th day up to 19th days of gestation. To evaluate the teratogenic effect of the drug, the following parameters were investigated, mortality rate, growth parameters, morphological and skeletal malformation. In addition, the oxidative stress markers were evaluated.

MATERIALS AND METHODS

All the experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CU/I/S /103/17).

Drug Used

Pantoprazole Tablets produced by PHARAONIA PHARMACEUTICALS (PHARO PHARMA) - Alexandria - A.R.E. Each tablet contains 40 mg of pantoprazole sodium sesquihydrate. The tablets were soluble in water.

Animal Housing

Healthy adult female and male rats (*Rattus norvegicus*), Wistar strain with an average age of approximately 2 to 2.5 months and weighing170-180 grams were obtained from the animal house of the Faculty of Veterinary, Cairo university- Egypt and used for experimentation.

The virgin female rats were mated with the male rats (Two females caged together with one male) overnight under standard conditions (21 ± 1°C, 60±20% humidity, and 12 h/12 h light/dark cycle). The first day of gestation was determined by the presence of sperm in the vaginal smear. [8]

Experimental procedure and dosing

In the present investigation, the recommended dose for human is 40 mg/daily. The dose was modified to suit the weight of rats. [9]

Ten pregnant rats, served as control group, was received equivalent amount of distilled water from the 6th day to 19 day of gestation, daily during gestation. Ten females treated group was received orally 4.11 mg/ Kg day of pantoprazole.

Growth observations

On the 19th day of gestation, all pregnant rats were weighted and dissected and uterus weighted then opened to remove fetuses with their placenta. Fetuses and placentas were removed from the uterus then weighted and evaluated for any external malformations (exencephaly, cleft palate, abdominal hernia, polydactyl, open eyelid, etc.) was performed under a dissecting microscope and their weights, mortality rate (resorbed or still birth) and growth parameters. The weights of the pregnant rats were recorded at the 6th and 19th GD and the % of change in maternal weight through the gestation was calculated as % change in maternal weight = (wt. of 20th day- wt. of 1st day / wt. of 20th day) x 100. In addition to, post-implantation loss index calculated. [10]

Post-implantation loss index= No. of implantation sites- No. of live fetuses/ No. of implantation sites x100.

Skeletal examination

Fetuses were preserved in 95% ethyl alcohol and were stained with double staining of fetal skeletons for cartilage (Alcian blue) and bone (Alizarin red) solution. [11]

Oxidative stress investigation

Autopsy samples that were taken from the placenta and liver of mother rats and fetuses in various groups were put away at - 40◦C for oxidative stress examination. Piece of each tissue were weighted and homogenized in 10 mmol/L phosphate buffer saline (PBS) as 10 % (W/V) at pH 7.4. The homogenates were centrifuged and the supernatants were taken for the estimation of: Glutathione Reduced (GSH), Catalase (CAT), Superoxide Dismutase (SOD) and Lipid Peroxidatio (MDA) by calorimetric method using reagent kits obtained from Bio Diagnostic (Egypt). [12-15]

Statistical analysis

All the values were presented as means (µ) ± standard errors of the means (S.E.M) comparison between two different groups was carried out using the independent student T test. GraphPad Software InStat (version 2) was used to carry out the statistical tests.

RESULTS

Effect of pantoprazole on pregnant rats

The pregnant rats treated orally with 4.11mg/Kg (group B) of pantoprazole during gestational period (6th - 19thday) revealed no external symptoms of toxicity. No mortality cases were recorded and we didn't score any dead case through the experimental duration.

The corrected mother body weight gain of the treated group was decreased than that of the control group but not significant (P› 0.05) (Table 1).

Table 1:

Group Item	Control (A)	Treated 4.11mg/Kg (B)
Corrected weight gain (C.W.G)	20.79±10.19	9.05±2.83
Uterus Weight (U.W)	36.21±2.61	24.54±3.10[a]
Placenta weight (P.W)	0.545±0.008	0.429±0.013[a]
post-implantation (Po.I)	1.25±1.25	19.48±9.31
pre-implantation (Pr.I)	27.75±7.08	27.11±6.84
Fetus weight (F.W)	2.97±0.094	1.71±0.067[a]
Viable fetus No. (VF)	7.5±0.734	7.0±0.966

Values are expressed as Mean ± SEM. The statistical differences were analyzed by independent samples T test. a= P ≤ 0.05 compared with control.

The uterus from control group revealed normal distribution of the implanted fetuses between the two horns (Fig.1) while the uterus of treated pregnant rats treated showed asymmetrical distribution of fetuses in the two uteri horns (Fig.1), resorption sites (late &early embryonic resorption) were observed (Fig.1) and completely early resorbed uterus also revealed that called pinpoint hemorrhagic implantation sites (Fig.1).

The average weight of placenta of all treated pregnant rats group was significantly (P≤ 0.05) decreased as compared to control (Table 1).

Moreover, there was a significant decrease in uterus weight (Table 1), non-significant increase in post-implantation loss index and decreased in pre-implantation loss index after comparison with the control group (Table 1) were observed.

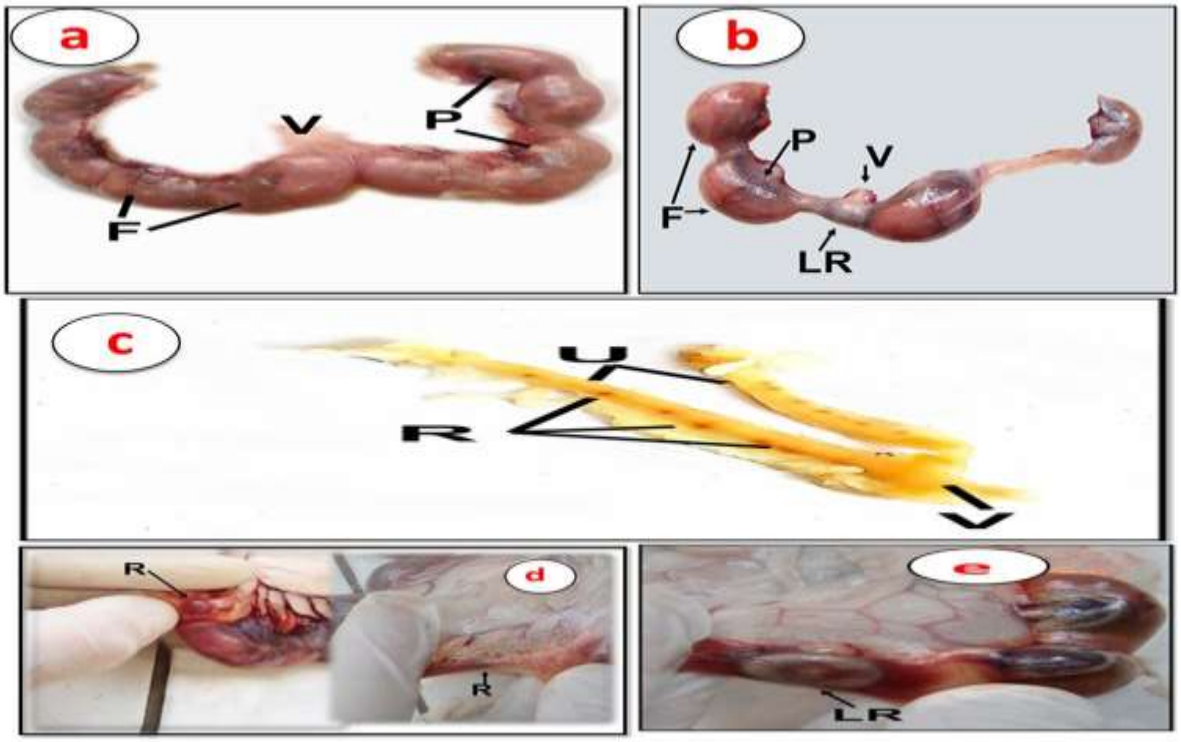

Figure 1: Photographs of uterus of pregnant rat at the 19th day of gestation.

From control group showing: **(a)** Normal symmetrical distribution of fetuses (F) in the two uteri horns. From treated group showing: **(b)** Asymmetrical distribution of fetuses in the two uteri horns with resorbed site (arrow). **(c)** Uterine horns showing pinpoint hemorrhagic implantation sites (early resorption= R). **(d)** Uterine horns showing clearly visible embryonic early resorption sites(R). **(e)** Uterine horns showing clearly visible embryonic late resorption sites (LR).

Effect of pantoprazole on fetuses

The fetuses of the control mothers have normal morphology, normal body weight (Fig.2) and appeared straight dorsally. Maternal administration of pantoprazole caused growth retardation represented by a decrease in fetal body weight (Fig.2). There was a significant (P≤ 0.05) reduction in fetus weight of treated group when compared with the control group (Table 1). There was a non-significant decrease (p > 0.05) in number of a live fetus of the pregnant rats that treated with 4.11 mg/Kg of pantoprazole when compared with the control group (Table1).The malformations found in fetuses of treated group are summarized in hematoma (red patches at different parts of the body) and whitish spots just under the left axilla (Fig.3).

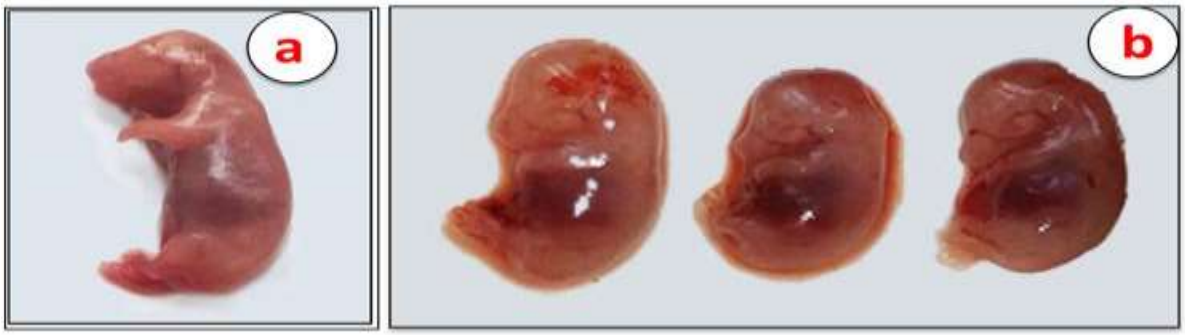

Figure 2: Photographs of fetus of mother at 19th day of gestation

From control group (a) showing fetus exhibited normal morphology with correct size and length. Treated mother with 11.4 mg/Kg showing (b) growth retarted Fetuses.

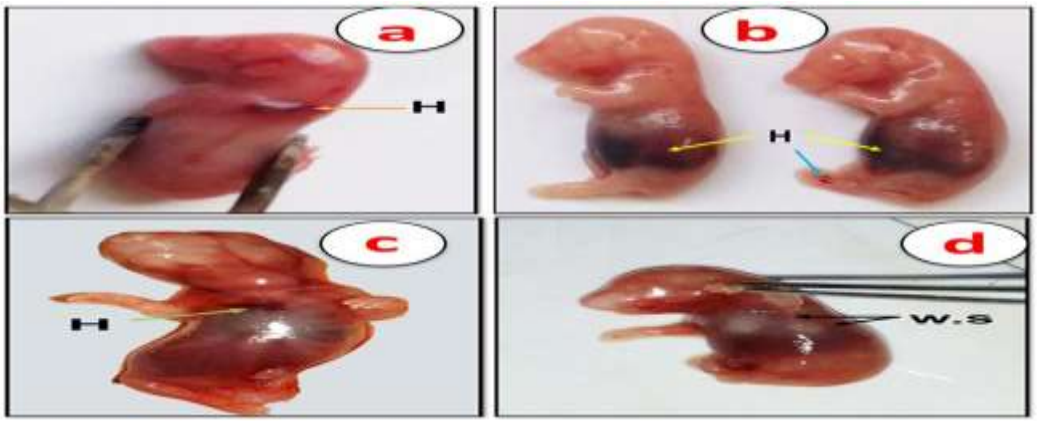

Figure 3: Photographs of fetus of mother at 19th day of gestation

Treated mother with 11.4 mg/Kg showing: **(a)** Dorsal Hematoma (Orange arrow). **(b)** Ventral Hematoma (yellow arrows) **and** Hematoma at tail (Blue arrow). **(c)** Hematoma at intra thoracic cage (Green arrow)**. (d)** Whitish spots just under the left axilla (Black arrows)

Skeletal anomalies

At the 19th day of gestation, the cleared cartilage and bone preparations of control rat fetuses have designated that all parts of the axial skeleton, skull, vertebrae and ribs as well as appendicular skeleton comprising the fore and hind limbs, pectoral and pelvic girdles, ossification process has been obviously completed (Fig. 4). On the other hand, fetuses maternally treated with 4.11mg/Kg of pantoprazole showed high percentage of incomplete ossification of all cranial bones (64.4%) from the examined fetuses (Fig.5), and the ribs anomalies represented in wavy , curved, rudimentary ribs, incomplete ossification degree and high percentage of un-ossified ribs (49.3%). (61.8%) of the sternum showed unossified sternebrae (Fig.6). In addition, girdle and limbs showed incomplete degree of ossification.

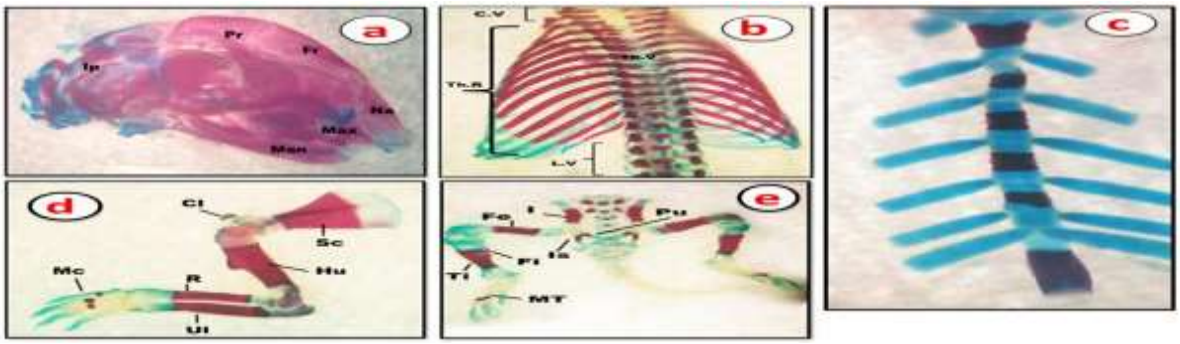

Figure 4: Photographs of the control fetal skeleton. (Alcian blue & Alizarin red stain).

a) **Skull bones**. **Showing** complete ossification of the cranial bones. Fr= frontal, Pr = parietal, N= nasal, Mx= maxilla, Ma= mandible, IP= interparietal. b) **Ribs and vertebral column. Showing** complete ossification. C.V= cervical vertebrae, Th.V= thoracic vertebrae, Th R= thoracic rib, L.V= lumbar vertebrae. c)**The sternum. showing** complete ossification of sternbrae bones. d) **Pectoral girdle and Forelimb**. **Showing** complete ossification. Cl= clavicle, Sc= scapula, H= humerus, U= ulna, R= radius, MC= metacarpals. e) **Pelvic girdle and Hind limb**. **Showing** complete ossification. I= ilium, IS= ischium, P=pubis, Fe= femur, Ti= tibia, Fi= fibula and MT= metatarsus.

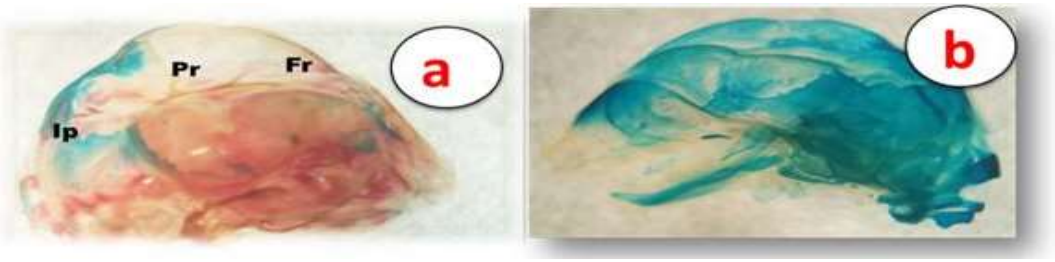

Figure 5: Photographs of the skull bones from treated group with 4.11 mg/Kg. Showing:

. **(a)** Incomplete ossification of all cranial bones. b) Unossified cranial bones

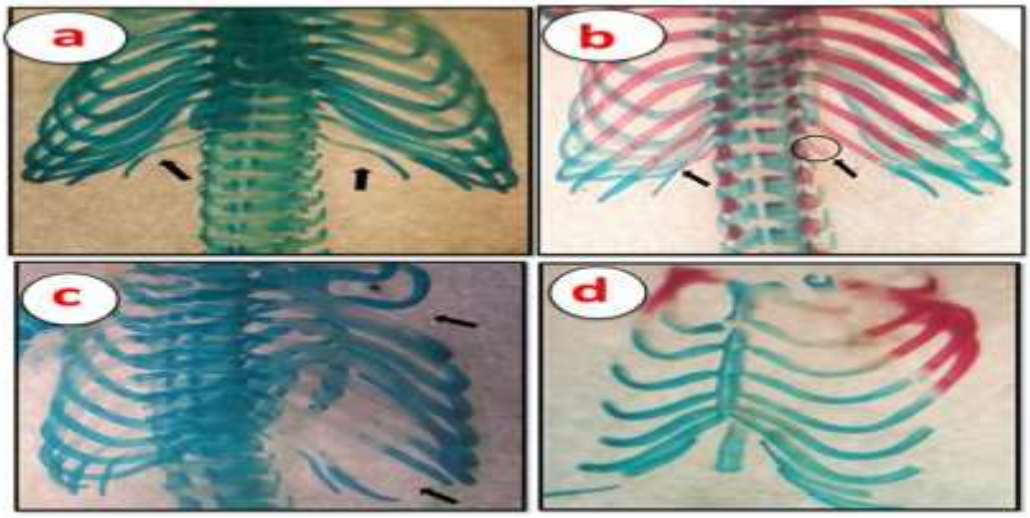

Figure 6: Photographs of the ribs and vertebral column from treated group with 4.11 mg/Kg. **Showing: a)** un ossification Curved ribs. (b) Incomplete ossification Wavy ribs and rudimentary ribs. (c) Costal separation. (d) Unossified sternbrae bones.

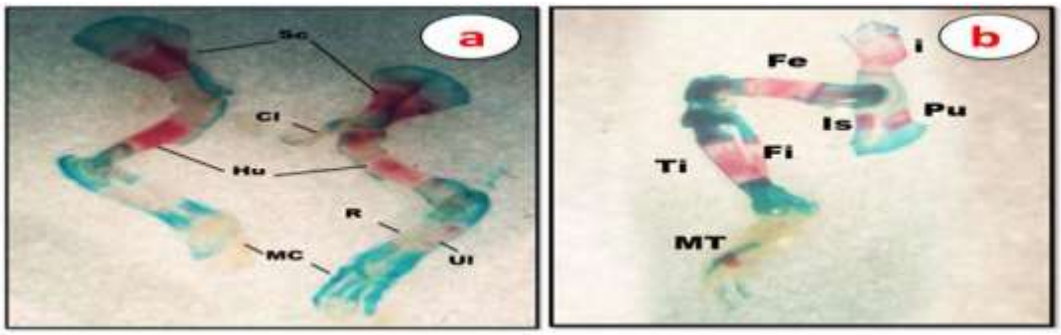

Figure 7: Photographs of fetuses from treated group with 4.11 mg/Kg.

Showing: (a) incomplete ossification pectoral girdle and Forelimb**. (b)** Incomplete ossification Pelvic girdle and Hind limb.

Oxidative stress observations

Reduced glutathione (GSH) is an important intracellular antioxidant and redox potential regulator that plays a vital role in cellular protection from damage by free radicals, peroxides and toxins. CAT serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. In addition the level of MDA was elevated as an indicator of lipid peroxidation and SOD quenches superoxide anion, thus its activity inhibits the overall colorimetric reaction.

1- Placenta tissue

There was significant reduction (P≤ 0.05) in the level of GSH was observed when pregnant rats treated with 4.11 mg/Kg of pantoprazole during gestation compared with the control group, non-significant elevation in the catalase activity and MDA were observed and significant reduction in the level SOD during gestation compared with the control group (Table2).

2- Maternal liver tissue

Oral administrated of 4.11 mg/Kg pantoprazole during gestation revealed significant decrease in the level of GSH, no significant decrease in the levels of antioxidant enzymes CAT and no significant increase in the levels of MDA was recorded when compared with control group. However, significant decrease (P≤0.05) in the level of SOD when compared with the control group (Table 3).

3- Fetal liver tissue

The content of GSH was decrease insignificantly in the hepatic tissue of fetuses treated with pantoprazole 4.11 mg/Kg. The fetuses of pregnant rats orally administrated with 4.11 mg/Kg and showed significant (P≤ 0.05) decrease in the activity of CAT and significant increase in the MDA antioxidant enzyme level. In addition to significant reduction in the SOD levels when compared with the control group (Table 4).

Table 2: Showing effect of pantoprazole on placenta tissue.

Group parameter	GA (control)	GB (4.11 mg/Kg)
GSH (U/g)	0.46±0.074	0.0511±0.0063[a]
CAT (U/g)	0.053±0.003	0.0567±0.0053
MDA (U/g)	0.62±0.057	0.74±0.059
SOD (U/g)	42.28±2.19	25.72±4.71[a]

Values are expressed as Mean ± SEM. The statistical differences were analyzed by

ANOVA followed by independent samples T test. a= P ≤ 0.05 compared with control.

Table 3: Showing effect of pantoprazole on liver of pregnant rats at 19th day of gestation.

Group parameter	GA (control)	GB (4.11 mg/Kg)
GSH (U/g)	0.373±0.038	0.24±0.043[a]
CAT (U/g)	0.157±0.017	0.098±0.0233
MDA (U/g)	1.27±0.36	1.54±0.086
SOD (U/g)	113.05±18.61	26.62±4.03[a]

Table 4: Showing effect of pantoprazole on liver of fetuses at 19th day of gestation.

Group parameter	GA (control)	GB (4.11 mg/Kg)
GSH (U/g)	0.38±0.086	0.355±0.032
CAT (U/g)	0.15±0.012	0.056±0.001[a]
MDA (U/g)	0.315±0.066	5.16±0.163[a]
SOD (U/g)	39.8±2.77	21.36±3.07[a]

DISCUSSION & CONCLUSION

Heartburn is a normal consequence of pregnancy, occurring in nearly two-thirds of women. Symptomatic GERD during pregnancy should be managed with lifestyle modifications and dietary changes. Antacids are considered the first-line medical therapy. If symptoms persist, any of the Histamine2-receptor antagonists can be used. These antagonists do not cause infantile sexual defects. Proton-pump inhibitors are reserved for women with intractable symptoms or complicated reflux disease. [16]

FDA classify the pantoprazole as category (B), the animal reproduction studies do not show any fetal risk. But there are no enough clinical trials or studies are available regarding the safety of pantoprazole use during pregnancy in woman. [17] The present study is carried out to evaluate the teratogenic potential of the proton pump inhibitor drug (Pantoprazole) on the female rats and their offspring orally administrated with (4.11mg/kg) during the gestation.

The safety of using pantoprazole which is newer PPI in pregnancy was proven in some animal experiments. [18] In our study, oral administration of Pantoprazole to female rat from 6th day up to 19th days of gestation did not reveal any signs of maternal toxicity as vaginal bleeding also no dead cases were observed. While the study showed a significant reduction in the placenta weight and fetal weight, high incidence of the resorption and high percent of the hematoma (dark spot) at different parts of the body (dorsal, ventral, intra thoracic cage and tail) also whitish spots just under the left axilla appeared on the fetal body as an external malformation when compared with the control. In addition, there was non-significant reduction in the maternal corrected body weight and non-significant increase in the percent of post-implantation loss were observed. The previous finding may be occurred due to the direct action of the drug or its metabolites on fetuses which can transfer via the placental barrier.

A study conducted in the 1960s reported that embryonic exposure to antacids during the last trimester of pregnancy would carry the risk of congenital malformations. In animal studies, cimetidine has a weak antiandrogenic effect in animals, in the form of smaller size of male genital system. [19] However, ranitidine has no such effect in animals. [20]

According to FDA, all PPIs are categorized as class B drugs except omeprazole due to its fetal toxicity which is classified as a class C drug. 12 birth defects as anencephaly and hydranencephaly were reported to FDA in pregnant women undertaken omeprazole. [19] Other researchers reported taking 20-60 mg omeprazole/day has no risk of fetal congenital anomalies even if taken from the first trimester. [21-23] Regarding lansoprazole, animal studies using doses exceeding 40 times the recommended human dose have no harm on fetus or fertility.

Skeletal malformations are clinically important as they are associated with severe disability and may cause death. [24] Incomplete ossification of the fetal skeleton may be a reason for fetal growth retardation and the consequential decrease in fetal weight. Un- ossification of several bones may be due to alternations in calcium metabolism or deviations in calcitonin levels in the developing fetus, leading to weak bone development. [25] The present study suggested that the oral administration of pantoprazole to the pregnant rat induced a broad variety of incompletely ossified, unossified skull bones, appearance of costal separation, incomplete ossification of the sternum, incompletely ossified, unossified bones of fore and hind limbs and absence of ossification of metacarpals and metatarsals.

While ROS are produced in large quantities continuously throughout life, both from mitochondrial and cytosolic elements, antioxidant defense mechanisms are vital for cellular homoeostasis. [26] Antioxidant activity is at the very least a continuous two stage process, with several potent

antioxidants such as SOD, catalase and glutathione peroxidase (GPx) providing first-line defenses by neutralizing superoxide and hydrogen peroxide. [27]

Growing fetuses are more sensitive to oxidative stress especially at the period of early organogenesis. Oxidative stress and redox related signaling are vital to normal developmental processes such as progression to the blastocyst stage, neuronal differentiation, and digit formation. In these events, inability to increase oxidative stress leads to abnormal developmental sequences and dysregulation. [28]

Oxygen in large quantities might be injurious to the fetus exceeding the fetal ability to neutralize these superoxide radicles, eventually leading to fetal damage. [29, 30] Once the Utero-placental circulation is well-formed, the oxidative stress is more tolerable by the fetus that develops a stronger and more stable antioxidant system. Basic principles of teratogenesis state that a teratogen must cause a specific malformation through a specific mechanism during a period in which the conceptus is susceptible to said mechanism. [31]

In current study, oral administration of pantoprazole during gestation may be induced oxidative stress damage via some non-enzymatic antioxidant defense system showed depletion in reduced glutathione (GSH), enzymatic antioxidants defense system showed reduction in the level of superoxide dismutase (SOD), increase of the activity catalase (CAT) in placental tissues but was decreased in hepatic tissues of mothers and their fetuses and the elevation of the level of malondialdehyde (MDA) in both pregnant rats and their fetuses was observed. All of these prove the teratogenic effect of the pantoprazole. Thus, we suggest the need for a great caution to handle pantoprazole especially during pregnancy.

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