TERATOGENIC EFFECT OF TRIMETHOBENZAMIDE: 
AN EXPERIMENTAL STUDY

G. Bayhan¹, I. Askar², A. Ketani³
Dicle University, Medical School, Department of Obstetrics and Gynecology, Diyarbakir, Turkey¹
Dicle University, Medical School, Department of Plastic and Reconstructive Surgery, Diyarbakir, Turkey²
Dicle University, Medical School, Department of Anatomy, Diyarbakir, Turkey³

ABSTRACT
In the experimental and clinical studies, it was reported that many pharmacological agents had teratogenic effects. There has been no sufficient explanation about the effect of trimethobenzamide, which is an antinauseant agent commonly used in Turkey. Thirty Wistar albino female rats, 3-month-old, weighing 200-250 g, were used to search the teratogenic effect of trimethobenzamide. In estrus, female rats were bred to singly housed males overnight. On the morning, the female rats with positive vaginal lavage for sperm were accepted as in the first gestational day (gd) 0. Sperm-positive females were divided into five groups, including six rats, by collecting randomly. In the first trimester (gd 6-15), in the control group (n=6), normal saline 0.5 ml was given subcutaneously; in the experimental groups, trimethobenzamide (Emedur®) was given subcutaneously, in dose of 17, 34, and 51 mg/kg/day, respectively. At death (gd 20), all live fetuses were evaluated for external, visceral, and skeletal abnormalities. No congenital abnormality was macroscopically seen. There was no developmental abnormality. However, a dose dependent developmental retardation was seen in bone and cartilage tissue, and a dose reduction in fetal body weight. The developmental retardation was prominent especially in vertebrae, and extremities.

Introduction
It was shown clinically and experimentally that pharmacological agents had teratogenic effects. A pharmacological agent, trimethobenzamide, is one of the most commonly used antinauseant agent during pregnancy and chemotherapy (Kousen, 293). Trimethobenzamide which is frequently used in the first trimester of pregnancy has borderline significance due to its own teratogenic effect (Milkovich and van den Berg, ‘76). It was associated with 2.6 percent serious congenital defects in the offspring at one year and in 5.8 percent at 5 years among 193 pregnancies (Milkovich and van den Berg, ‘76).

There has been no experimental study that clearly show the teratogenic effect of trimethobenzamide. We searched teratogenic effect of trimethobenzamide, using Wistar albino rats.

Materials and Methods
In this experimental study, 30 Wistar albino female rats, weighing 200-250 g, were used. Rats were divided into groups including five rats. In estrus, each group was bred to singly housed males overnight. On the morning, the female rats with positive vaginal lavage for sperm were accepted as in the first gestational day (gd) 0. Sperm-positive females were divided into five groups, including six rats, by stratified randomization. In the first trimester (gd 6-15),
in the control group (n=6), normal saline 0.5 ml was given subcutaneously; in the experimental groups, trimethobenzamide (Emedur®, Dogu, Istanbul, Turkey) was given subcutaneously, in dose of 17, 34, and 51 mg/kg/day, respectively. At gd 20, all pregnant rats were sacrificed with ether anesthesia. The maternal body, liver and uterus, and fetal body was evaluated for weight. All fetuses were taken using cesarean section. At death (gd 20), all live fetuses were evaluated for weight, external, visceral, and skeletal abnormalities. Soft tissues, such as, skin, eye, visceral and connective tissue were taken away from the skeleton. Later on, all fetuses were kept in a solution of alcohol 95% to fix tissues. To stain tissues better, all these fetuses were exposed to microwaves (Imperial V8505T Microwave Oven - 2450 mHz, 550 W), for three minutes. To prevent temperature higher and higher, two glasses of cold water were placed in microwave oven. All specimens were kept in acetone solution overnight to separate adipose tissue. Later on, all specimens, in double staining solution (alcian blue+alizarin red S), were kept at 37 °C in drying oven for a period of four days. Then, specimens were washed with tap water, and placed into a solution of KOH 1% for two days. And, specimens were treated with the solutions of KOH 1%, including glycerin 50% and 80% sequentially. Glycerin 100% was used to get specimens transparent. Thymol was added to glycerin to prevent cultivation of fungi (Inouye, '76). After staining, all specimens were examined under stereomicroscope (SMZ-ZT Nikon).

**Statistical Analysis:** The values of maternal weights were evaluated with Kruskal-Wallis ANOVA and Man Whitney U test (p < 0.05). The values of fetal body weights were evaluated with ANOVA test (p < 0.05).

**Results and Discussion**
At death (gd 20), there was no congenital abnormality macroscopically seen. The measurement of maternal body, liver and uterus weight, and fetal body weight was given on Table 1, 2, 3, and 4 respectively. And only fetal body weights of experiments were given in the table.
Fig. 1. In the control group, normal development of fetal skeleton (alcian blue-alizarin red S, x0.67).

Fig. 2. In Group II, developmental retardation of skeleton, especially bone and cartilage tissue, was seen in vertebral bodies, and both upper and lower extremities (alcian blue-alizarin red S, x0.67).

Fig. 3. Staining changes showed developmental retardation of bone and cartilage tissue in both upper and lower extremities, ribs, vertebral bodies and spinous processes (alcian blue-alizarin red S, x0.67).

Fig. 4. Developmental retardation is more advanced in bone and cartilage tissues (alcian blue-alizarin red S, x0.67).

In the control group, there was no developmental abnormality (Fig. 1), dose-dependent developmental retardation of bone and cartilage tissue was seen in the experimental groups. In the first experimental group (Group II), developmental retardation of bone and cartilage tissue was seen in vertebral bodies and extremities of all rats, and four rats had thin diaphragm (Fig. 2). In the second experimental group (Group III), all rats had developmental retardation of bone and cartilage tissue in vertebral bodies and spinous processes, both upper and lower extremities, and ribs (Fig. 3). In the third experimental group (Group IV), developmental retardation of bone and cartilage tissue, which had skeleton stained darker, was seen in both upper and lower extremities, vertebral bodies and spinous processes, and ribs (Fig. 4).

It was demonstrated that administration of trimethobenzamide, subcutaneously, during organogenesis in Wistar Albino rats, resulted in dose-dependent developmental toxicity, especially in bone and cartilage tissue, in our experimental study. The effects of trimethobenzamide were observed at doses which are one to three times as much as the human therapeutic dose, estimated at 9-17 mg/kg/day. Commercial forms, as produced by Dogu Co., have...
coated tablets, ampules, and suppositories of 200 mg trimethobenzamide, and total daily dose of trimethobenzamide is 600 – 1200 mg in adults (Ommaty, ‘93). In our experimental study, it was found out that the maximum dose of trimethobenzamide led to the most severe developmental retardation in bone and cartilage tissue.

Four rats with thin diaphragm were observed, in the first experimental group (trimethobenzamide 17 mg/kg/day). This may also increases incidence of diaphragmatic hernia. However, it further needs to investigate thinness of diaphragm in future studies.

Maternal body weight, hepatic weight and uterine weight was evaluated but it was found that there was no difference between all groups. However, fetal body weight had an inverse relation to dose of trimethobenzamide administered. Thus, the developmental toxicity could be explained with a reduction in fetal body weight and a retardation in bone and cartilage tissue, depending on dose of trimethobenzamide.

By using teratogenicity model which were preferred by Tyl et al., it was shown that trimethobenzamide, administered during organogenesis, increased incidences of dose-dependent malformations (Tyl et al., ‘88).

Development of fetal bone and cartilage tissue was evaluated depending on stain uptake and length of bone after fetal rat skeleton was stained with alcian blue and alizarin red-S. An increase in blue color and a decrease in red color shows developmental retardation in bone and cartilage tissue. Thus, developmental retardation can easily be observed especially in vertebrae, ribs, upper and lower extremities. A decrease in red color presented a reduction in fetal ossification, in ribs, corpora and spinous processes of vertebrae, upper and lower extremities. Because normally fetal ossification centers was red colored.

In conclusion, we thought that fetuses of mothers who were treated with trimethobenzamide might have anomalies especially in vertebrae and extremities. But, it further needs investigations to determine whether it has borderline significance of teratogenicity, or not.

REFERENCES