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Original Article

THE TERATOGENIC EFFECT OF DIETHYLENE GLYCOL (DEG) ON FETUS MORPHOLOGY IN WHITE MICE (*MUS MUSCULUS* L.)

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ABSTRACT

Objective: Diethylene glycol is listed in the Inventory of Cosmetic Ingredients as a solvent, viscosity controller, and fragrance. Most of diethylene glycol's toxicity is caused by ingesting the contaminated product. This study aims to determine the effect of a teratogen caused by diethylene glycol on mice fetuses.

Methods: In this study, 20 mice were used, which were divided into four groups. Diethylene glycol was given with three variations of doses 1662.5, 3325, and 6650 mg/kgBW during the organogenesis period, day 6-15. The effects of teratogens observed were maternal body weight, fetal body weight, and number of fetuses, which were analyzed using one-way ANOVA, as well as morphological and skeletal abnormalities, which were analyzed descriptively.

Results: Based on the results of the study, there was a significant difference in the body weight of the mice given diethylene glycol and the control group ($p<0.05$). There was no significant difference in body weight and the number of fetuses in the control and treatment groups ($p>0.05$). On the results of fixation of Bouins' solution, defects in the form of hemorrhage, resorption sites, and slow growth were found. At the same time, the results of the fixation of alizarin red solution found defects in the sternal, nasal, caudal, metacarpal, metatarsal, and phalanges bones.

Conclusion: The conclusion based on the research is that diethylene glycol has the potential to provide teratogenic effects on mouse fetuses.

Keywords: Diethylene glycol, Teratogen, Morphology, Skeletal, Toxicity

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INTRODUCTION

Diethylene glycol (DEG; CAS number 111-46-6) is a liquid industrial chemical that is used in the dehydrogenation of natural gas, production of polyurethane and unsaturated polyester resins, petroleum solvents, humectants, a key ingredient in explosives and some refrigeration formulations. Diethylene glycol proved to be an excellent solvent and a substitute for glycerin. However, due to the lack of scientific data and the number of poisoning cases that have occurred, diethylene glycol is not approved for use in food [1].

Recently, cases of poisoning have reoccurred due to contamination of ethylene glycol and diethylene glycol in children's syrup. This case affects children aged 6 mo to 18 y, and there has been an increase in cases, especially in the last two months. In October 2022, 189 patients had been reported, dominated by ages 1-5 y. This poisoning case is suspected to be caused by ethylene glycol and diethylene glycol contamination found in mixed solvents. The syrup preparations suspected of containing ethylene glycol and diethylene glycol contamination probably came from four additional ingredients, namely propylene glycol, polyethylene glycol, sorbitol, and glycerin/glycerol, which are not hazardous materials or are prohibited from being used in the manufacture of syrup preparations. According to BPOM, the recognized national standard for the safe threshold or Tolerable Daily Intake (TDI) of ethylene glycol and diethylene glycol contamination is 0.5 mg/kg body weight per day [2].

During pregnancy, pregnant women can experience various health problems and complaints that require medication to treat them. The impact of drug use on pregnant women varies widely. This depends on drug and gestational age when the drug is consumed. Pregnant women who consume drugs containing teratogenic compounds can cause abortion in an early stage of pregnancy or cause congenital defects if consumed during the organogenesis period [3].

In general, the use of drugs during the period of organogenesis can have a direct impact on the baby because some drugs can cross the placenta. Transfer of drugs through the placenta generally takes place by simple diffusion so that the drug concentration in the mother's blood and placental blood flow will greatly determine the transfer of drugs through the placenta. The properties of drugs that can cross the placenta are fat-soluble drugs that will make the drug diffuse easily across the placenta into the fetal circulation, drugs that are not ionized, drugs that are not bound to protein, and drugs with a molecular size<500 Dalton [4].

In previous studies, it was found that diglycolic acid (DGA) was accumulated by the kidneys after administration of diethylene glycol, where there were concentrations of DGA and 2-hydroxyethoxyacetic acid (HEAA) in kidney tissue when kidney toxicity occurred. HEAA is found in higher concentrations than DGA in blood, where it is the metabolite responsible for metabolic acidosis [5]. DGA and HEAA are thought to be the primary metabolites causing kidney damage caused by diethylene glycol. In teratology studies, it is suspected that DGA is a teratogen for diethylene glycol and metabolic acidosis because it can increase the teratogenic effect by increasing the parental pH gradient and increasing the dose of glycolic acid for the fetus [6].

In teratology, information on the potential developmental toxicity of diethylene glycol is less extensive and detailed than for ethylene glycol. Given this and the limitations of other studies with diethylene glycol, *in vivo*, tests on mice at oral doses investigated the teratological effect of diethylene glycol. This study aims to observe the teratogenic effect of diethylene glycol administration on the morphology and skeletal structure of white mice fetuses. Observations were made by looking at the side effects of diethylene glycol on the morphology of white mice fetuses.

MATERIALS AND METHODS

Material

Diethylene glycol, aqua dest, alcohol swab, filter paper, bouins solution, alizarin red, husk, food and water.

Animal preparation

The experimental animals were acclimatized for ten days at the Animal House of the Faculty of Pharmacy, Andalas University. During the acclimatization process, the experimental animals will be adapted to the practical environment. Food and drink are given in moderation, body weight is weighed every day, and behavior is observed. The experimental animals will be declared healthy if the animals do not have weight fluctuations of more than 10% and show normal behavior [7]. The experimental animals used have met the ethical approval by the Research Ethics Committee of the Faculty of Pharmacy, Andalas University, with approval number 08/UN.16.10. D. KEPK-FF/2023.

Mating of experimental animals

In the estrus phase, mice are mated with males in a ratio of 1:4 (polygamous mating). Mice are nocturnal animals whose mating process is carried out at 4 in the afternoon. The following day, a vaginal plug was observed, which was characterized by a yellowish plug in the vagina which was a mixture of female seminal vesicle secretions and hardened male ejaculate. Vaginal mice that have vaginal plugs will be considered to be in gestation zero period. Mice that are already pregnant can be separated, and those that are not pregnant can be re-mated with male mice [8].

The administration of test preparations

The test preparation was administered orally with a sonde once a day during the organogenesis period, on days 6-15, with a dose distribution of 1662.5, 3325, and 6650 mg/kgBW. The dose is selected based on the LD50 value of the diethylene glycol, where the dose used is 1/2, 1/4, and 1/8 of the LD50. The mice group was divided into four groups, each consisting of 5 mice. Determining the size of the research sample used the provisions of the World Health Organization (WHO) with a minimum sample size of 5 test animals per group. The control group was only given aquadest and mice staple food. The test group will be given a test preparation with a predetermined dosage for mice and the mice's staple food.

Laparotomy

Laparotomy was performed on day 18. This procedure is a surgical incision in the abdominal tissue for access to the abdominal cavity which is performed aseptically [9]. For observation, mice were humanely killed, uterine contents examined, and fetuses evaluated for morphology and skeletal defects. The laparotomy surgical procedure will incise the abdominal muscle layers one by one to access the abdominal cavity and eventually reach the fetus. The fetus is removed from the placenta and the uterus, and placenta are cut.

Morphological fixation and observation

After the laparotomy stage is carried out, observations will be made on fetal weight and the number of developing fetuses from each group. Each fetus must be cleaned from the fluid and blood adhering to it with filter paper before being weighed and the weighing data recorded. Then proceed with the fixation of the number of fetuses from one parent with Bouins solution for 14 d until they are yellow and hard. After 14 d, the fetus is removed and dried. Then, observations were made on the outside of the fetus, namely the ears, eyes, tail and cleft palate. Another part is soaked with an alizarin red solution and left for three days until the fetus becomes transparent and the red bone skeleton is observed. Examination results in the form of structure, morphology, number, size, bone position and degree of staining were recorded and compared with the control group [10]. The morphological assessment of fetus was done by our team research.

Data analysis

Examination data for maternal and fetal body weight data as well as the number of fetuses, were statistically analyzed using the one-way Analysis of Variance (ANOVA) method during the 0^{th} to 18^{th} day of pregnancy. The alpha value for statistical significance is taken at 0.05. Before the ANOVA test is carried out, the normality and data homogeneity tests will be carried out. Examination data from the ANOVA test that met the significance ($p<0.05$) would be further analyzed using Duncan's Multiple Range Test. Data on examination of the type of defects observed, and observations of fixation with bouins solution and alizarin red were analyzed descriptively with the control group and related literature.

RESULTS

The effect of diethylene glycol administration on maternal body weight, fetal body weight, and number of fetuses in mice

Weighing of the mice from day one to one day before the mice were about to laparotomy to see the differences in the weight gain of the mother mice between the control group and the group was given diethylene glycol at a dose of 1662.5; 3325; and 6650 mg/kgBW. The mice's weight gain during pregnancy can reflect the mother's health and nutrition during pregnancy [11].

Table 1: The effect of diethylene glycol administration on maternal body weight, fetal body weight, and number of fetuses

Parameter	Group				p-value
	Control	Dose 1662.5 mg/kgBW	Dose 3325 mg/kgBW	Dose 6650 mg/kgBW	
Mother's mice weight	24.86±1.04[a]	17.90±1.05[b]	17.90±1.10[b]	17.94±2.43[b]	<.001
Fetus's weight	1.05±0.12	0.77±0.11	0.78±0.19	0.70±0.10	.095
Fetus's number	12.26±1.82	11.00±0.71	10.40±1.82	10.20±2.59	.220

Notes: The mean data with different superscripts in the rows showed a significant difference ($p<0.05$) based on duncan's multiple-range tests analysis

The mean difference between the initial body weight of the mother mice and the final body weight in the control group, the dose group of 1662.5, 3325, and 6650 mg/kgBW, respectively was 24.86±1.04, 17.9±1.05, 17.9±1.10, 17.94±2.43. It can be seen in table 1 that the average body weight of the parent group given diethylene glycol was lower than that of the control group. The mother's weight gain indicates the development of the fetus, the increase in the size of the placenta, the increase in amniotic fluid and the amniotic membranes of mice. The greater the weight gain, can provide the potential for more fetuses to be born [12].

Based on the results of the one-way ANOVA statistical analysis, it can be seen that the teratogen diethylene glycol's effect on the mother mice's body weight showed a significant difference where a significant value of<0.001 was obtained. Then continued with Duncan's multiple area test with the result that there was a significant difference between the control group and the dose group 1662.5, 3325, and 6650 mg/kgBW. While between groups of dose, there is no significant difference.

Fetal weight loss is the mildest form of the teratogenic effect of a compound, where weight loss is caused by reduced transfer of nutrients during fetal development. Fetal weight loss is an indicator of obstacles during fetal growth due to disturbances in the processes that underlie growth (cell division, metabolism and synthesis) [13, 14]. The average fetal weight in the control group, 1662.5; 3325; and 6650 mg/kgBW group dosage respectively were 1.05±0.12; 0.77±0.11; 0.78±0.19; 0.70±0.10. In the statistical test, the average data on fetal weight in each mouse was used and the results of the normality test and normal homogeneity test were obtained ($p>0.05$). Based on the results of the ANOVA test on the effect of the teratogen diethylene glycol on fetal body weight, it showed that there was no significant difference between groups with a significant value of 0.095 which can be seen in table 1.

The average number of mice fetuses in the control group, 1662.5, 3325, and 6650 mg/kgBW group dosages,respectively were 12.60±1.82; 11±0.71; 10.40±1.82; 10.20±2.59, which can be seen in

table 1. In the statistical test, it was found that the data on the number of mice fetuses was normally distributed and homogeneous. However, in the one-way ANOVA test there was no significant difference (p=0.220) between the administration of diethylene glycol and the number of fetuses. The number of fetuses is influenced by the age of the mother, genetics and the condition of the mice when mated; therefore, diethylene glycol does not make a significant difference in the number of fetuses in mice.

The effect of diethylene glycol on the morphology and skeletal of the mice fetus

The site of resorption is an undeveloped fetus, characterized by the presence of red lumps in the uterus where the fetus is embedded [15]. The resorption site describes the number of fetal deaths experienced in mice. At the time of laparotomy, resorption sites were found in the group given diethylene glycol at a dose of 3325 mg/kgBW for one fetus and 6650 mg/kgBW for one fetus. The resorption site found was dead when it was removed from the uterus. In the group given diethylene glycol, growth retardation was found in the fetuses, where some fetuses had smaller size and weight than other fetuses in the same parent. The number of fetuses that experienced growth in the group given diethylene glycol was 1662.5 mg/kgBW for six fetuses, 3325 mg/kgBW for 6, and 6650 mg/kgBW for nine fetuses.

Morphological abnormalities in the form of hemorrhage were not found in the control group, however found in the group given diethylene glycol (fig. 1). The amount of hemorrhage that occurred in the treatment group tended to increase with increasing doses. Based on our study, in the 1662.5 mg/kgBW group dosage, there were three hemorrhagic fetuses. In the 3325 mg/kgBW group, the dosage had five hemorrhagic fetuses, and in the 6650 mg/kgBW dosage group there were six fetuses experiencing hemorrhage. Hemorrhage that occurs is found on the chest, back, stomach, head and hands.

For skeletal defect, in the control group, it can be observed that there are six bones in the sternum. The manubarium, sternebrae 1, sternebrae 2, sternebrae 3, sternebrae 4 and xiphoid. Based on the observations in fig. 2, it can be seen that in the 3325 mg/kgBW dosage group, the xiphoid bone is almost perfectly divided, and there is a lack of number-only three sternebrae bones. Likewise,with the 6650 mg/kgBW dosage group, it was clear that the xiphoid bone had been completely split in half and there were only three sternebrae bones. In both the 3325 mg/kgBW and 6650 mg/kgBW dosage groups there was a difference in the thickness of the sternebrae, where the bones in the diethylene glycol group were much thinner and smaller.

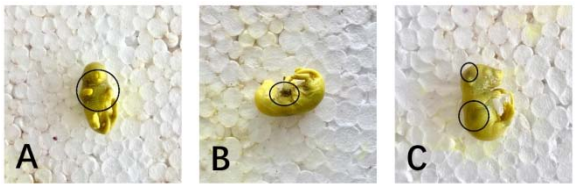

Fig. 1: Hemorrhagic fetus on (A) DEG dosage group 1662,5 mg/kgBW, (B) DEG dosage group 3325 mg/kgBW, and (C) DEG dosage group 6650 mg/kgBW

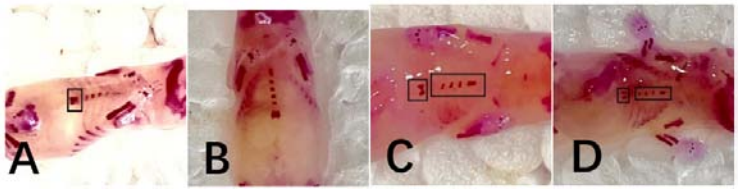

Fig. 2: Normal sternum on (A) Control group, (B) DEG dosage group 1662,5 mg/kgBW; Defects sternum on (C) DEG dosage group 3325 mg/kgBW, (D) DEG dosage group 6650 mg/kgBW

For nasal bones, there was a deformity (fig. 3) where the nasal bones were shorter than the mandibular bones, while in the control group the two bones were parallel. However, this defect only occurs in one fetus in DEG dosage group 6650 mg/kgBW out of all the fetuses used in the study. Meanwhile, for caudal bone, in the group given diethylene glycol at a dose of 3325 mg/kgBW and 6650 mg/kgBW, we can't find the caudal bone (fig. 4). Whereas for the dosage group of 1662.5 mg/kgBW, the fetus had the same normal caudal bone as the control group.

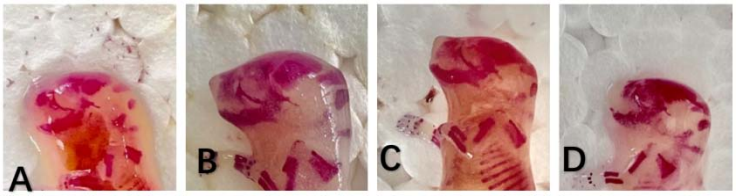

Fig. 3: Normal fetus nasal bones in (A) Control group, (B) DEG dosage group 1662,5 mg/kgBW, (C) DEG dosage group 3325 mg/kgBW; Deformity fetus nasal bones in (D) DEG dosage group 6650 mg/kgBW

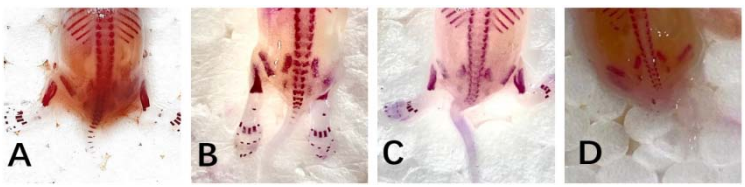

Fig. 4: Normal caudal bone in (A) Control group, (B) DEG dosage group 1662,5 mg/kgBW; Defect caudal bone in (C) DEG dosage group 3325 mg/kgBW, (D) DEG dosage group 6650 mg/kgBW

In the upper and lower limbs, the hands and feet, there are incomplete bones in the fingers (fig. 5 and 6). In the group given diethylene glycol, the average metacarpal, metatarsal and phalange bones were incomplete. Based on the literature, mice have eight metacarpal bones, eight metatarsal bones, and 16 phalanges found in the hands and feet. From observations of the 1662.5 mg/kgBW group, it was found that the fetus only had seven metacarpals and incomplete phalanges. In the 3325 mg/kgBW group, there were fetuses with incomplete five metatarsals and phalanges. In the 6650 mg/kgBW group, there was a fetus with four metatarsals and no phalanges. This defect occurs due to a delay in bone growth which is characterized by a decrease in the number of bony segments of the metacarpus, metatarsus and phalanges.

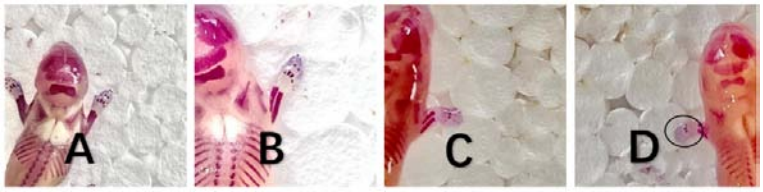

Fig. 5: Normal metacarpal bone in (A) Control Group; Defect metacarpal bone in (B) DEG dosage group 1662,5 mg/kgBW; (C) DEG dosage group 3325 mg/kgBW, (D) DEG dosage group 6650 mg/kgBW

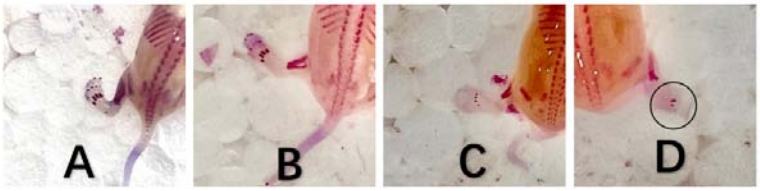

Fig. 6: Normal metatarsal bone in (A) Control group; defect metatarsal bone in (B) DEG dosage group 1662,5 mg/kgBW; (C) DEG dosage group 3325 mg/kgBW, (D) DEG dosage group 6650 mg/kgBW

DISCUSSION

The organic molecule diethylene glycol has the chemical formula $(HOCH_2CH_2)_2O$ is a hygroscopic liquid that is colorless, almost odorless, and tastes sweet. Diethylene glycol is frequently used as a solvent. Due to the lack of scientific data and the number of poisoning cases, diethylene glycol is not approved for food use [1]. A teratogenic test is a test that provides information regarding fetal abnormalities that arise as a result of administering the test preparation during the period of fetal organ formation (organogenesis period). This information includes defects in the exterior of the fetus (morphology), soft tissue and fetal skeleton [16].

Diethylene glycol is an organic compound containing hydroxyl groups and can be classified as an alcohol group. Alcohol given early in pregnancy can damage the placenta so that it can directly interfere with the process of embryo development. Alcohol absorbed into the fetus can inhibit nutrition, especially folic acid and amino acids from mother to child so that it becomes a factor for low body weight. Alcohol compounds given repeatedly during pregnancy can cause hypoxia. Hypoxia can be teratogenic by reducing oxygen in the metabolic process. Low oxygen in the blood results in inhibition of nutritional intake from the mother to the fetus [17]. While nutrition is important and can influence mother and fetus's health [18]. Based on study, the administration of diethylene glycol significantly decrease the weight of the mother mice ($p<0.05$), but it was not significant for fetal weight and number of fetuses ($p>0.05$).

On morphological observations, resorption sites were obtained, fetuses experiencing growth delays, and hemorrhages on the back, abdomen, chest, arms, and head. Internal abnormalities in the fetus are defects in the sternal, nasal, caudal, metacarpal, metatarsal, and phalangal bones. The resorption site describes the number of fetal deaths experienced in mice. Resorption sites were found in the group given diethylene glycol at a dose of 3325 mg/kg for one fetus and 6650 mg/kg for one fetus. Diethylene glycol is given during the organogenesis period, which means that the mice no longer have totipotent properties so that there is no repair of the damaged tissue so that the fetus cannot survive and no development occurs. In addition, diethylene glycol is known to cause metabolic acidosis which is a condition where the acid levels in the body are very high. This condition causes a decrease in blood pH which will disrupt cellular integrity and can destroy fetal tissue so that development does not occur [19].

Growth retardation or growth delays was also found in the fetuses that were given diethylene glycol. Some fetuses had lower size and weight than others in the same parent. In this case, it can be assumed that there is an imbalance of nutrients obtained by each fetus, which can be associated with hypertrophy that occurs in the group given diethylene glycol [20]. Hypertrophy is a condition with abnormal enlargement of mice's veins. Hypertrophy causes a shortage and uneven distribution of nutrients that enter the placenta, resulting in growth delays in the fetus. The uterus of mice given diethylene glycol showed hypertrophy in the veins [21].

Hemorrhage is the release of blood from the cardiovascular system, accompanied by the accumulation of body tissue. Based on this study, hemorrhage is found on the chest, back, stomach, head and hands. It is suspected that the bleeding was caused by repeated administration of diethylene glycol, so its concentration in the blood was quite high and disrupted the osmotic balance. Amniotic fluid in the body is isotonic under normal conditions, so the presence of foreign substances can cause changes in osmotic pressure. This imbalance causes pressure disturbances and fluid viscosity in the fetus, which differ between blood plasma and the intercapillary space or excess and intraembryonic fluid, resulting in bleeding or rupture of blood vessels [22].

Morphological abnormalities do not occur in all fetuses in the same group because there are genetic differences between individuals even though they come from the same parent. Some fetuses do not experience any defects at all, experience one disability, and experience more than one disability. Abnormalities that occur in the fetus are thought to occur due to the accumulation of diethylene glycol which is an agent that causes hypoxia. Diethylene glycol in the placenta causes nutritional barriers from the mother to the fetus due to the lack of oxygen needed for organ development, including mineral materials for bone growth (ossification) or the bone hardening process (calcification) [23].

Several studies have shown that excessive alcohol intake can lead to bone deformities and an increased risk of bone fragility, although

the relation to bone loss is still controversial. In addition, alcohol can reduce the quantitative distribution of Calcium (Ca) and Phosphorus (P), which are essential elements in bone formation [24].

CONCLUSION

This study concluded that there was a significant difference ($p<0.05$) in the mother's body weight between the control group and the group given diethylene glycol. However, there was no significant difference ($p>0.05$) in the body weight and number of fetuses. There were morphological abnormalities in the form of hemorrhage on the back, abdomen, chest, hands, and head in the fetuses; and there were skeletal abnormalities in the bones of the sternum, nasal, caudal, metacarpal, metatarsal, and phalanges of the fetus that were given diethylene glycol.

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AUTHORS CONTRIBUTIONS

AA, EB, and YY designed the study, JA carried out the fieldwork, EB and JA wrote the manuscript, and the final version was reviewed and approved by all authors.

CONFLICT OF INTERESTS

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

REFERENCES

1. European Commission. Opinion on diethylene glycol (Dec); 2006.
2. BPOM RI. Fourth information results of BPOM supervision of drug syrups suspected of containing ethylene glycol (EG) and diethylene glycol (DEG) contaminants; 2022.
3. Prince MK, Daley SF, Ayers D. Substance use in pregnancy. Pearls Publishing; 2023.
4. Feghali M, Venkataramanan R, Caritis S. Pharmacokinetics of drugs in pregnancy. Semin Perinatol. 2015;39(7):512-9. doi: 10.1053/j.semperi.2015.08.003, PMID 26452316.
5. Besenhofer LM, McLaren MC, Latimer B, Bartels M, Filary MJ, Perala AW. Role of tissue metabolite accumulation in the renal toxicity of diethylene glycol. Toxicol Sci. 2011;123(2):374-83. doi: 10.1093/toxsci/kfr197, PMID 21804082.
6. Ballantyne B, Snellings WM. Developmental toxicity study with diethylene glycol dosed by gavage to CD rats and CD-1 mice. Food Chem Toxicol. 2005;43(11):1637-46. doi: 10.1016/j.fct.2005.05.005, PMID 15979775.
7. National Research Council. Guide for the care and use of laboratory animals; 2011.
8. Byers SL, Wiles MV, Dunn SL, Taft RA. Mouse estrous cycle identification tool and images. PLOS ONE. 2012;7(4):e35538. doi: 10.1371/journal.pone.0035538, PMID 22514749.
9. Rajaretnam N, Okoye E, Burns B. Laparotomy. Pearls Publishing; 2023.
10. Clarke GS, Gatford KL, Young RL, Grattan DR, Ladyman SR, Page AJ. Maternal adaptations to food intake across pregnancy: central and peripheral mechanisms. Obesity (Silver Spring). 2021;29(11):1813-24. doi: 10.1002/oby.23224, PMID 34623766.
11. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. Endocr Rev. 2006;27(2):141-69. doi: 10.1210/er.2005-0011, PMID 16434511.
12. Mousa A, Naqash A, Lim S. Macronutrient and micronutrient intake during pregnancy: an overview of recent evidence. Nutrients. 2019;11(2). doi: 10.3390/nu11020443, PMID 30791647.
13. Kaleelullah RA, Garugula N. Teratogenic genesis in fetal malformations. Cureus. 2021;13(2):e13149. doi: 10.7759/cureus.13149, PMID 33692919.
14. Flores LE, Hildebrandt TB, Kuhl AA, Drews B. Early detection and staging of spontaneous embryo resorption by ultrasound biomicroscopy in murine pregnancy. Reprod Biol Endocrinol. 2014;12:38. doi: 10.1186/1477-7827-12-38, PMID 24886361.
15. Alwan S, Chambers CD. Identifying human teratogens: an update. J Pediatr Genet. 2015;4(2):39-41. doi: 10.1055/s-0035-1556745, PMID 27617116.
16. Dejong K, Olyaei A, Lo JO. Alcohol use in pregnancy. Clin Obstet Gynecol. 2019;62(1):142-55. doi: 10.1097/GRF.0000000000000414, PMID 30575614.
17. Putri AMN, Chandra DN, Werdhani RA, Bardosono S. Relevant topics for education modules on healthy eating during pregnancy in the context of Indonesia. Int J App Pharm. 2020;12;Special Issue 3:58-62. doi: 10.22159/ijap.2020.v12s3.39475.
18. Dillasamola D, Almahdy A, Desri A, Diliarosta S. Teratogenic effect test of yoghurt on the fetus of white mice. Mus musculus. 2018;5(1):28-32.
19. Paradis F, Wood KM, Swanson KC, Miller SP, McBride BW, Fitzsimmons C. Maternal nutrient restriction in mid-to-late gestation influences fetal mRNA expression in muscle tissues in beef cattle. BMC Genomics. 2017;18(1):632. doi: 10.1186/s12864-017-4051-5, PMID 28821223.
20. Ezeuk VC, Ataman JE, Grillo DB. Toxic effects of antituberculosis drugs (isoniazid and rifampicin) on fetoplacental unit of Wistar rats: a morphological, histological and biochemical study. J Clin Exp Tox. 2019;3(1):1–6.
21. Setyawati I, Yulihastuti DA. Reproductive performance and development of the fetal skeleton of mice after administration of Young pineapple fruit extract. J Vet. 2011;12(3):192-9.
22. Bocheva G, Boyadjieva N. Epigenetic regulation of fetal bone development and placental transfer of nutrients: progress for osteoporosis. Interdiscip Toxicol. 2011;4(4):167-72. doi: 10.2478/v10102-011-0026-6, PMID 22319250.
23. Foger Samwald U, Knecht C, Stimpfl T, Szekeres T, Kerschan Schindl K, Mikosch P. Bone effects of binge alcohol drinking using prepubescent pigs as a model. Alcohol Clin Exp Res. 2018;42(11):2123-35. doi: 10.1111/acer.13874, PMID 30120836.