ORIGINAL ARTICLE

The Evaluation of Teratogenic Effects of Tramadol on Mouse Fetuses

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ABSTRACT

Introduction: Tramadol belongs to the group of quasi-narcotic compounds and is used as an analgesic drug with central effects in the treatment of moderate-to-severe acute pain (after surgery and trauma) and chronic cancer pain, and chronic non-cancer pain, for example, due to osteoarthritis. Tramadol is a weak μ-opioid receptor agonist and its analgesic activity is mainly based on serotonin reuptake inhibition. This drug is a weak reuptake inhibitor of norepinephrine. It is contraindicated in patients with a history of seizure disorders, and its use increases serotonin syndrome. Due to the occurrence of fetal complications, in this study, the probability of the teratogenic effects of tramadol on the mice embryo was investigated.

Method: Pregnant mice were fed a tramadol dose of 1-3-9-27-50-100 mg/kg during the 7th to 9th day of pregnancy, and were anesthetized with isoflurane on the 15th day of pregnancy. The fetuses were removed to assess the number of miscarriages, fetal and placental weights, fetal length and placental diameter, liver size and uterine diameter, and histopathological examination, and were studied for developmental toxicity.

Results: The results of statistical analysis showed that tramadol significantly reduced fetal weight and fetal length after exposure to 1-3-9-27-50-100 mg/kg concentrations. However, at a dose of 27 mg/kg, fetal weight and length did not change significantly. The weight of the placenta increased significantly at a dose of 27 mg/kg, and the weight and diameter of the placenta decreased significantly at a dose of 100 mg/kg, and there was a significant increase in uterine horn diameter and endometrial thickness compared to the control group. Also, histopathological examination showed that tramadol causes skeletal anomalies and pathological disorders in the brain, liver, abdominal wall, and placenta.

Conclusion: As a result of the research, the use of tramadol during pregnancy causes anomalies in fetal development and histopathological changes in the process of brain and liver development. Therefore, it is recommended to be careful in taking this drug during pregnancy.

Keywords: Tramadol, Anomaly, Embryonic-Fetal Toxicity, Developmental Toxicity,mousefetus

INTRODUCTION

Teratology is an important branch of toxicology that studies congenital anomalies and birth defects (1). Teratogen is a factor that directly and indirectly affects the growth of the fetus by causing side effects and causes tissue damage and permanent structural and partial irreversible defects[1]. Therefore, when taking drugs during pregnancy, miscarriage and teratogenicity effects should be considered. In addition to infectious and genetic diseases and physical conditions and factors, drugs and chemicals can be teratogenic and lead to the birth of infants with congenital defects[1].

Birth defects and congenital disorders and anomalies are similar terms describing structural, behavioral, functional, and metabolic disorders appearing at the time of birth[1]. Skeletal defects at birth are among the main disorders affecting almost 21% of all infants. These defects are the main cause of mortality among people aged less than 65 and are among the important causes of disabilities (1). Anomalies occurring during the formation of skeletons, for instance during the organ formation period, may lead to the complete or partial absence of one or a part of the structure of an organ or changes in its natural form. Most anomalies develop from the third to eighth weeks of pregnancy[1].

Experience has shown that many different medicinal compounds can cause similar defects if all are prescribed at a specific time in pregnancy[2,3]. These similarities led to the theory of the existence of multiple mechanisms of teratogenesis[2,3]. Teratogenic substances can act through one or more of these mechanisms. Some mechanisms can produce fewer cells or fewer cellular products or prevent the natural differentiation of cells and counteract cellular functions. Cell death or tissue necrosis is a common symptom of most chemical or physical damages to the developing fetus. Although cell death associated with the number of cells above the physiological limit does not lead to malformation, if a large cell mass is destroyed, it can affect the differentiation and physiological functions of cells[2,3].

Tramadol: Tramadol was first discovered and synthesized in 1962 by a German company, Grunenthal GmbH, for the treatment of pain. However, it was introduced in 1977 to the market under the name "tramadol" and was introduced in the United States after 1995[4]. In recent years, tramadol has been used to treat a variety of osteoarthritis pains, chronic cancer pains, neuropathic pains, postoperative pains, and pains caused by neuropathic allergy to morphine[4].

Tramadol received the US Food and Drug Administration (FDA) label in March 1995 for the management and control of moderate to severe pain[5]. Tramadol is a central analgesic that is used to prevent and treat moderate to severe pain under acute or chronic conditions[6]. A toxicological study of both pure substance and tramadol formulation using pouched rats, rats, guinea pigs, rabbits, and dogs of both sexes according to the methods for oral, intravenous, intramuscular, anal, intraperitoneal, and subcutaneous administration showed that LD50 results showed that oral administration of 300 mg/kg in rats, 350 mg/kg in pouched rats, more than 800

mg/kg in guinea pigs, between 450-600 mg/kg in rabbits, 450mg/kg in dogs was without mortality[6]. Tramadol is recognized by the FDA as a class C drug in pregnancy, and it is excreted in human milk and is not recommended for use during lactation[7].

In general, the use of tramadol in pregnancy is limited and no association has been reported with major organ defects or minor structural defects. Besides, no observations have been made in toxicological studies on the teratogenic effects of tramadol on the female reproductive system[8].

This study aimed to investigate the teratogenicity of tramadol in developing fetuses of pouched rats. In particular, it investigates whether tramadol can reach the fetus through the pregnant mother and slow down the growth or development or abort the developing fetus. The insights from this study may lead to a reconsideration of tramadol use during pregnancy.

EXPERIMENTAL DESIGN

Devices and tools: The devices and tools used in this study included a binocular optical microscope (Model M3, Zeiss Co., Germany), a manual rotary micrometer (Jung, Germany) a digital scale for weighing rats with an accuracy of 0.1 g (ASD, Japan), an analytical scale with an accuracy of 0.0001 g (Shimadzu, Japan), a distillation device, a table time, a complete surgical set (forceps, scalpels, scissors, a dissection tray), laboratory glassware (beakers, petri dishes, a graded desiccator cylinder) (Merck, Germany), micrometer graticule (graded eye lens) (KaraProject, Iran), paraffin glass molds (Merck, Germany) an oven for gradual melting of solid paraffin and also placing bath 1 and 2 inside it (Stark Eliwellwpc, UK), glass containers for coloring (Merck, Germany), metal shelves for keeping rats, a caliber for measuring fetal C-R length, and lam and lamella pair diameter (KaraProject, Iran), a digital camera, computers, printers, and latex gloves.

Compounds: The compounds and substances used in this study were tramadol (Ramofarmine, Iran), medical alcohol (96%) (Merck, Germany), medical alcohol (1005) (Merck, Germany), toluene (Merck, Germany), paraffin with a melting point of 50-60 degrees (Merck, Germany), Entelen adhesive (Merck, Germany), Zylen (Merck, Germany), pyric acid (Merck, Germany), stick glassil(Merck, Germany), formalin (Merck, Germany), hematoxylin solution (KaraProject, Iran), eosin (KaraProject, Iran) gelatin (KaraProject, Iran), isofloran (for anesthesia), distilled water (Kimia, Iran), physiological serum (Kimia, Iran), and insulin syringes (Saha, Iran).

Animals: Animals used in this study were pouched laboratory rats purchased from Royan Research Institute (Tehran, Iran). All rats (with a bodyweight of 25-30 g) were kept in standard conditions in a room with a constant temperature of 25 °C and a 12-hour light/dark cycle with water and food. All experiments were performed following ethical standards and protocols approved by the Animal Testing Supervision Committee affiliated with ShahidBeheshti University of Medical Sciences (Tehran, Iran).

The rats were divided into seven groups, each group with four adult female rats (aged 10 to 12 weeks) that had no mating experience, and were selected based on the observation of the vaginal plaque and the preparation of vaginal smears of pregnant rats. These groups include the control group (that received normal saline) and the experimental groups (which took tramadol orally at concentrations of 1, 3, 9, 27, 50, and 100mg/kg on days 7 to 9 of pregnancy). The rats were anesthetized with isoflurane on the 15th day of pregnancy. Using the laparotomy technique, the uterus was visible and the number and location of the fetuses were determined, and then their weight and crown-rump length (CR) were measured. Careful examinations were performed on each fetus to determine external abnormalities, and then half of the fetuses were stained with hematoxylin or eosin. The presence of bone malformations was examined with an optical microscope and with the naked eye.

Experimental Group 1: The group included 6 groups of 4 adult female rats (aged 10-12 weeks) without the experience of pregnancy and the uterus of each mouse carried 10 to 12 fetuses. The weight of the rats was measured in grams, and tramadol was administered orally at different doses of 1, 3, 9, 27, 50, and 100 mg/kg orally on days 7, 8, and 9 of pregnancy based on their body weight. The experiments were performed on the rats on the 15th day of pregnancy.

Control Group: This group included 4 rats that were bred in the absence of completely normal pregnancy (under the same conditions considered for the rats in the other groups) and this group was used as the basis for comparison between the other groups.

On the 15th day of pregnancy, the pregnant rats were anesthetized and then described, and the fetuses and placentas, if present, were removed from the uterus and ovaries and weighed after washing with saline. The diameter of the placenta and C-R of the fetuses were measured by a vernier scale. Afterward, microscopic sections of the samples were prepared for microscopic and histological studies and the histological changes on the fetuses were examined microscopically. They were microscopically placed. The obtained results were given to the computer as raw data. The results were reported in terms of mean ± SD. The intergroup statistical signification

Table 1: The time table for sequences of the events in the staining procedure

Sequence of events	Stage	Material	Time
1	Dewaxing of paraffin	Toluene	5-6 minutes (if necessary repeated)
2	Hydration	Alcohol 100 Alcohol 90 Alcohol70 Alcohol50 Distilled water	5 min 5 min 5 min 5 min 5 min
3	Staining	Hematoxylin Washing with water Alcohol50 Alcohol70 Eosin	3-5 min 5 min 5 min 5 min 5 min
4	Dehydration	Alcohol 90 Absolute Alcohol	30 second 30 second
5	Clearing	Toluene	5-10 minutes

nce was checked using Graph Pad Prism (Model 8). The collected data were analyzed using one-way ANOVA, and the post-test data were analyzed by Dunnett's test at the significance level of 0.05 ($p<0.05$). Table 1 shows the time table for sequences of the events in the staining procedure:

RESULTS AND DISCUSSION

Morphologic studies

1. Brain damage with tissue changes compared to the control group
2. Having a larger liver compared to fetuses in the control group
3. Umbilical hernia in the fetus
4. Observing blood-filled placentas compared to placentas in the control group
5. Fetal growth disorders compared to the fetuses in the control group
6. The development of skeletal abnormalities in comparison with the fetuses in the control group

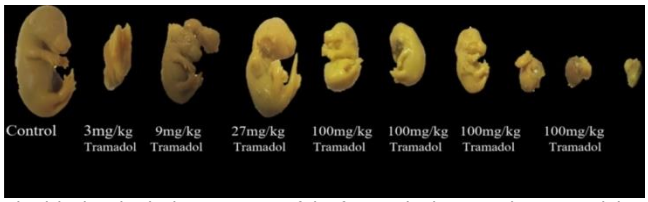

Fig. (1) The physical appearance of the fetuses in the control group and the groups receiving tramadol

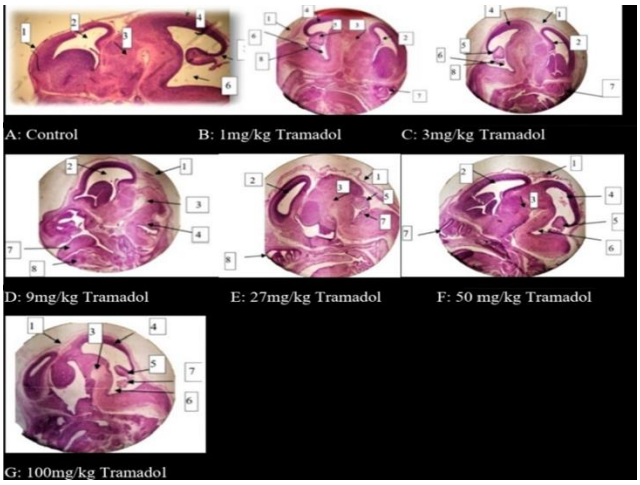

Fig. (2) A comparison of brain photomicrograph in the longitudinal incision of the fetuses in the control group and the tramadol receiving group (Microscope with X4 magnification) [(1) Primary cortex, (2) lateral ventricles, (3) diancephalus, (4) mesencephalon, (5)metencephalon, (6) the fourth ventricle, (7) eye, and (8) cerebellum]

As can be seen, the primary cortex of the brain of the animals in the experimental groups is uneven compared to the control group. With increasing tramadol doses at 3, 9, and mg/kg, the primary cortex of the brain is completely uneven and deformed compared to the control group and is not uniform in the diancephalus. Besides, the location of themesencephalon, metencephalon, and the fourth ventricle is not clear, and there are some tissue malformations compared to the control group. The lateral ventricles occupy a smaller space than the control group. At 50 mg/kg dose of tramadol, the primary cortex is uneven and the lateral ventricles are uniform compared with the control group. Besides, at the dose of 100 mg/kg, the diancephalus is shrunk and this shrinkage can be seen in all sections of the brain with an abnormal form as compared to the animals in the control group.

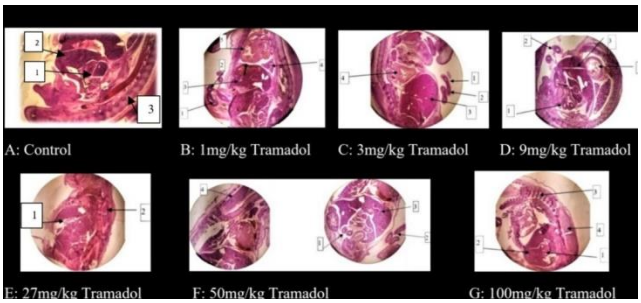

Fig. (3): A comparison of fetal abdominal surface photomicrograph in the control group and the tramadol receiving group (Microscope with X4 magnification) [(1) intestinal loop, (2) umbilical hernia, (3) the liver, (4) the lung, and (5) the heart]

As can be seen in the figure above, the teratogenic effect of tramadol at 1, 3, 9, and mg/kg in the fetus exposed to tramadol causes umbilical hernia and a larger liver (covering the entire abdominal space) in contrast to the fetus in the controlled group. The teratogenic effect of tramadol at 50 and 100 mg/kg in a fetus exposed to tramadol has resulted in a larger liver (the atrophied liver) compared with the control group. Furthermore, the fetuses in the experimental group show edema behind the vertebrae, resulting in the deformation of the vertebrae and the spine.

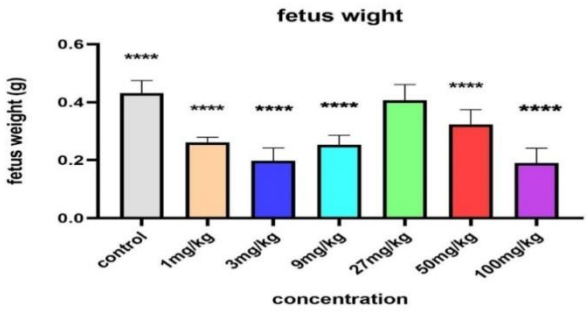

Fig. (4) A comparison of fetal mean weight at the tramadol concentrations of 1, 3, 9, 27, 50, and 100 mg/kg

As can be seen, the mean fetus weights have decreased significantly after exposure to the tramadol concentrations of 1, 3, 9, 27, 50, and 100 mg/kg compared to the control group ($p<0.0001$). However, the fetal weight in the group receiving 27mg/kg of tramadol did not change and showed no significant difference compared to the control group ($p>0.05$).

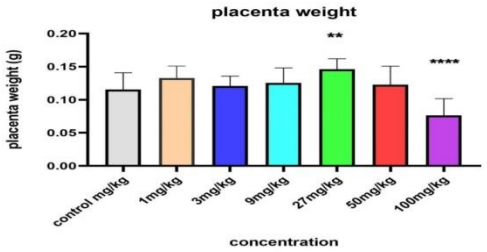

Fig. (5) A comparison of the mean weight of the placenta at the tramadol concentrations of 1, 3, 9, 27, 50, and 100 mg/kg

As it is shown, the mean weight of the placenta after exposure to 27 mg/kg of tramadol increased significantly compared to the control group ($p<0.0027$). Besides, the mean weight of the placenta after exposure to the 100 mg/kg of tramadol decreased significantly compared to the control group ($p<0.0001$). However, in the groups receiving 1, 3, 9, and 50 mg/kgof tramadol, the placenta weight did not change and there was no significant difference compared to the control group ($p>0.05$).

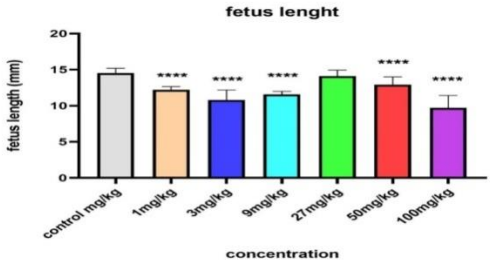

Fig. (6) A comparison of the fetus lengths at the tramadol concentrations of 1, 3, 9, 27, 50, and 100 mg/kg

As can be seen, the fetus lengths were significantly reduced in the groups receiving 11, 3, 9, 50, and 100 mg/kg of tramadol compared to the control group ($p<0.0001$). However, in the group receiving 27 mg/kg of tramadol, the fetus's length did not change significantly ($p>0.05$).

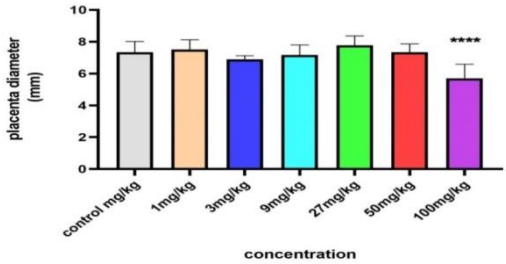

Fig. (7) A comparison of the placenta diameter at the tramadol concentrations of 1, 3, 9, 27, 50, and 100 mg/kg

The diameter of the placentas in the group receiving 100 mg/kg of tramadol decreased significantly compared to the control group ($p<0.0001$). However, the placenta diameter in the groups receiving 1, 3, 9, 27, and 50 mg/kg of tramadol did not change significantly ($p>0.05$).

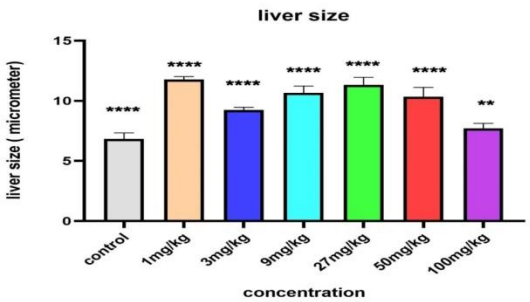

Fig. (8) A comparison of the liver size at the tramadol concentrations of 1, 3, 9, 27, 50, and 100 mg/kg

The liver size of the fetus increased significantly in the groups receiving 1, 3, 9, 27, and 50 mg/kg of tramadol ($p<0.0001$) besides the group receiving 100 mg/kg of tramadol showed in a significant increase in the liver size ($p<0.0019$) compared to the control group.

DISCUSSION

Previous studies in the literature have shown that teratogenic factors have different effects on the fetus depending on the dose, the route of exposure, and the time of exposure in short-term and long-term periods. Besides, individual conditions can result in different defects. The prescribed dose of the drug or the effect of the drug can cause irreparable damages (9).

Tramadol is an analgesic opioid with the central activity that has dose-dependent effects and is used to treat moderate to severe acute pain (after surgery and trauma) and chronic cancer and non-cancer pains such as osteoarthritis. It is a weak μ-opioid receptor agonist and its analgesic activity is mainly based on inhibition of serotonin reuptake. This drug is a weak blocker of norepinephrine reuptake (10). Patients with a history of seizure-related disorders are prohibited to use tramadol as and its use increases the incidence of serotonin syndrome (10). In pregnancy, the issue of fetal growth restriction cannot be ignored. It is clear that some factors, in addition to causing major and minor structural defects that are teratogens, can prevent fetal growth (11).

A study by BasmaEmadAbdulhoda et al. showed that tramadol administration during pregnancy in female rats of Sprague Dawley strainscaused profound structural abnormalities on the post-natal cerebellar cortex of the fetuses and on days 7, 14, and 21 after birth and was associated with oxidative stress evidenced by elevation of lipid peroxidation products and inhibition of antioxidant enzyme activities. In fact, enzymatic and structural changes were shown to have an oxidative effect on stress on the fetal cerebellum (12).

Faria et al. studied the toxicity effects of tramadol and tapentadol on the heart, brain, and lungs of Wistar rats and showed that tramadol and tapentadol increase protein oxidation in the lungs and heart and destroy brain neurons and cause damages to the lungs and heart (13).

Chomchai et al. reviewed the effects of recreational and unconventional use of drugs during pregnancy in animal studies and showed that tramadol consumed by pregnant rats caused oxidative stress in the fetus cerebellum. The human studies on Swedish infants

performed during six months showed that infants whose mothers took tramadol during pregnancy were diagnosed with genital abnormalities. It was shown that the mothers who received tramadol due to chronic pain in their lower back or shoulders during pregnancy and also a mother who took the drug during breastfeeding developed neonatal abstinence syndrome (NAS) (14).

Kalen et al. conducted a study on Swedish infants and showed that 96 infants had congenital anomalies, of which 70 infants had very severeanomalies. The average odds ratio for major defects after taking tramadol was 1.33 (1.05-1.70, 95% CI), the odds ratio for cardiovascular failure was 1.56 (1.04-2.29, 95% CI) and the odds ratio for disorders related to the lower limbs was 3.63 (1.05-6.89, 95% CI) which has increased significantly and this study showed that tramadol has teratogenic effects but the risk is moderate[15].

A study by Buthina et al. (2018) on pregnant rats found that fetuses exposed to tramadol at a dose of 20 mg/kg showed fetal toxicity in the early stages of pregnancy and all pregnant rats exposed to tramadol aborted fetuses, and the fetus was absorbed into the uterine endometrium[8].

Tramadol has been used in recent years to treat a variety of pains including osteoarthritis pain, chronic cancer pain, neuropathic pain, and postoperative pain[4]. It is a central analgesic drug first discovered and synthesized in 1962 by a German company, Grunthal GmbH, while it was marketed as tramadol in 1997[4]. The chemical name of tramadol is (±) -cis-2 - ((dimethylamino) methyl) -1 (m-methoxyphenyl) cyclohexanol, and its hydrochloride salt is a crystalline white powder with a structural resemblance to codeine[16]. Tramadol in its pharmaceutical form is a rheumatic mixture with two mechanisms of action available in the pharmaceutical market, a weak μ-opioid receptor agonist inhibiting the reuptake of norepinephrine and serotonin in the central nervous system, and the analgesic response of this compound is related to its dual mechanism[6]. Compared with NSAIDs, tramadol does not exacerbate blood pressure, does not irritate asthma, does not damage the gastrointestinal mucosa, and does not cause kidney failure or congestive heart failure, and unlike other opioids, does not lead to severe respiratory depression, histamine release, constipation, and severe addiction[17]. Non-prescribed and addictive use of the drug causes seizures, kidney and liver failure, cardiovascular problems, and death[17]. A toxicological study was done on both pure substance and medicinal formulation of tramadol on pouched rats, rats, guinea pigs, rabbits, dogs of both sexes. The drug was administered orally, intravenously, intramuscularly, anally, intraperitoneally, and subcutaneously. The LD_{50} values after the oral administration were 300mg/kg in rats, 350 mg/kg in pouched rats, more than 800mg/kg in guinea pigs, between 600-450 mg/kg in rabbits, and 400 mg/kg in dogs without mortality[6]. Tramadol was classified as a Class C pregnancy drug by the FDA[7]. Given that tramadol is a derivative of opioids, it can have destructive effects on the human fetus and infants, even if these effects are reversible and do not have a teratogenic nature. However, the risks of these drugs have not been ruled out, and the doctor will prescribe them when he or she feels the benefits of taking this group of medications outweigh the potential harms and if they are needed during pregnancy[7]. On the other hand, tramadol abuse and addiction is a very serious problem for women during pregnancy because it can affect the health of pregnant women and the healthy growth of their children due to the drug dependence created during treatment, the history of substance abuse in the patient, reassurance due to the availability of the drug in pharmacies, and the sense of security in its use due to the doctor's prescription for the patient's previous condition. Research into whether tramadol can reach the fetus through the pregnant mother and slow the growth or development of the fetus may lead to a reconsideration of tramadol use during pregnancy.

This study investigated the transgenicity of tramadol in developing fetuses of pouched rats. After conducting the relevant experiments, the results of this study were discussed in light of the findings of other studies in the literature.

Different organs and tissues of the developing fetus have different sensitivities to different types of substances and drugs. Some drugs exert their toxic effects on a specific part of the system, so to examine the effect of a substance or drug on the developing fetus, different sensitivities need to be considered.

The morphological/histological effects of different doses of tramadol on the fetus of pouched rats

Tramadol effects on fetal weight and crown-rump length (CR)

The fetuses were weighed and measured on the 15th day of pregnancy. The mean fetus weights decreased significantly after exposure to the tramadol concentrations of 1, 3, 9, 27, 50, and 100 mg/kg compared to the control group. However, the fetal weight in the group receiving 27 mg/kg of tramadol did not change and showed no significant difference compared to the control group. Tramadol appears to cause vasoconstriction, thereby reducing placental blood flow and subsequently reducing blood flow to the fetus's brain, and since drugs such as tramadol can cross the placental barrier, they have devastating effects on various fetal organs. According, the administration of tramadol to the pregnant mother during pregnancy can have a direct impact on fetal growth, reduce the number of cells in different organs, and have a negative effect on cell division and normal growth and development. This finding was consistent with some studies that considered fetal weight loss to be a sensitive and accurate indicator of growth retardation and attributed the decrease in blood flow to the uterine horn to fetal growth retardation (18). On the other hand, when the maternal organism is stressed, it may, in turn, affect the developing fetus, leading to growth retardation and resulting in fetal weight loss, and fetal weight loss is associated with reduced bone formation, leading to decreased body skeleton weight and moderate body weight loss[19]. Skeletal and visceral abnormalities may be considered a sign of growth retardation and lead to fetal weight loss[19].

Tramadol effects on placenta weight and diameter: The placenta grows rapidly in early pregnancy, but the fetus grows faster in the third trimester. As the gestational age increases, the placenta-weight ratio decreases[21].

The present study showed that the mean weight of the placenta increased significantly after exposure to 27 mg/kg of tramadol compared to the control group. Accordingly, tramadol easily crosses the placenta and reduces maternal-placental blood supply, resulting in the increased fetal need and compensatory enlargement of the placenta.

In the present study, in the groups receiving 1, 3, 9, and 50mg/kg of tramadol, the placenta weight did not change and there was no significant difference compared to the control group. However, the weight of the placenta reduced significantly in the group receiving 100 mg/kg of tramadol. Similarly, the placenta diameter was significantly reduced in the group receiving a dose of 100 mg/kg of tramadol compared to the control group, and other doses did not result in a significant difference with the control group.

Tramadol effects on the brain and abdominal area of the developing fetus: Buthina (2018) showed that pregnant rats that received 20 mg/kg of tramadol orally from 1-7 days of pregnancy lost all their fetuses due to prolonged exposure to opioid analgesics, its easy passage through the placental barrier, reduced blood flow, and insufficient nutrition to the fetus, leading to fetal toxicity[8]. Another study by BasmaEmadAbdulhoda et al. suggested that tramadol administration during pregnancy in female rats of Sprague Dawley strains caused profound structural abnormalities on the post-natal cerebellar cortex of the fetuses and on days 7, 14, and 21 after birth and was associated with oxidative stress evidenced by elevation of lipid peroxidation products and inhibition of antioxidant enzyme activities. In fact, enzymatic and structural changes were shown to have an oxidative effect on stress on the fetal cerebellum[12].

Chomchai et al. showed that tramadol did not affect pregnancy. However, their animal studies reported the oxidative damage of Purkinje cells of the cerebellum. Human studies also showed severe congenital defects, arterial and ventricular septum defects, and neonatal abstinence syndrome (NAS)[14].

Faria et al. studied the toxicity effects of tramadol and tapentadol on the heart, brain, and lungs of Wistar rats and showed that tramadol and tapentadol increase protein oxidation in the lungs and heart and destroy brain neurons and cause damages to the lungs and heart[13].

The experiments conducted in this study showed a delay in the normal development of the fetal abdominal area and an umbilical hernia at all tramadol doses, indicating a delayed differentiation because the closure of the abdominal area usually occurs between 14 and 14 embryonic days without any sign of an umbilical hernia on the 15th day of pregnancy; however, our findings showed an umbilical hernia in all the prescribed doses of tramadol.

This study showed brain tissue abnormalities in the fetuses due to the administration of tramadol, so that the brain shrinkage was increased in all areas of the brain in a dose-dependent manner. Substances that affect the fetal brain are often fat-soluble and low in molecular weight. These substances quickly cross the placenta and a rapid balance is established between the mother and the fetus. However, the drug accumulates in the fetus because the fetal enzymatic and renal functions are immature. Tramadol easily crosses the blood-brain and the placental barrier, and drugs are more slowly excreted in infants than in adults, causing damage to their liver[8].

As displayed in Fig. 8, the results of the study showed that the size of the fetal liver increased significantly compared to the control group, and even at a dose of 100 mg/kg there some liver degeneration, so prescribing tramadol to pregnant rats could cause growth abnormalities and histological changes in the process of fetal liver tissue development.

The present study also showed vertebral irregularities at tramadol doses of 27, 50, and 1000 mg/kg.In some parts of the spine, there was a complication of edema, such as behind the vertebrae, resulting in the vertebrae and spine deformation. Therefore, the abnormalities increased dose-dependently. Tramadol at doses lower than 27 mg/kg did not have negative effects on the fetal spine of pouched rats, while at higher doses it had devastating effects on the structure of the spine. These changes and injuries can be a function of the dose, time, and duration of tramadol intake.

The findings of the study also showed that 4 fetuses developed nodes in the uterine horn as a result of the cessation of growth and development in the early stages of development and degeneration of the fetus. Such abnormalities were observed at a dose of 3 mg/kg for one rate and a dose of 100 mg/kg for three rats.

A case of abnormal brain growth (exencephaly) was observed at a dose of 9 mg/kg and an abnormality in the formation of the hand was observed in the form of abnormal shortness (micromelia) with abnormalities in the foot at a dose of 27mg/kg.

The fetuses in the group receiving 100 mg/kg of tramadol showed symptoms such as the release of the limbs, the soft skeleton, and the twisted limbs in opposite directions.

Based on the observations made in this study, it can be suggested that immune system activity acts as a toxic agent in increasing immune cells such as macrophages for tramadol cleansing. As a case in point, at the tramadol dose of less than 27mg/kg, the placenta retains its structure based on its physiological function, but due to the easy passage of the drug through the placental barrier, there is a toxic induction in fetuses and weight loss. At a dose of 27mg/kg, the immune system recognizes tramadol as a harmful substance that induces toxicity and thus some reactions occur in the fetus to create a barrier to the tramadol passage, resulting in insignificant changes in the fetal weight compared to the control group. However, the placenta has a compensatory enlargement due to loss of efficacy; this enlargement may be due to the presence of immune cells. At tramadol doses higher than 27 mg/kg, the toxic induction is extremely high. For instance, at a dose of 100 mg/kg, there would be changes in the structure of the placental physiological function and its incompetence, because the weight and diameter of the placenta at this dose have a significant reduction compared to the control group.

Another point to note is that different doses of tramadol may have different effects on fetal weight since drugs such as tramadol can cross the placental barrier and have devastating effects on different parts of the fetus. In

this study, the weight-to-body ratio was not measured, and thus other organs may be more damaged.

In sum, our observations showed that tramadol in pouched mouse fetuses causes growth retardation and the appearance of abnormalities and histological changes in the development of liver tissue and abdominal contents, as well as changes in their brain tissue.

CONCLUSIONS

This study explored the effects of different doses of tramadol and its teratogenic impacts on pouched mouse embryonic tissue, and the results are summarized as follows:

1. Tramadol acted as a teratogen and has left many morphological and histopathological damages on the mouse fetus.
2. Clear abnormalities in different parts of the brain, exencephaly, reduction of fetal length and weight, delayed intrauterine growth of the fetus, liver enlargement, umbilical hernia, thickness of the endometrium and cross-section of the uterine horn, abnormalities in the hand, and the congestion of the placenta were important changes in the fetuses exposed to tramadol.
3. The use of tramadol is in pregnancy is a questionable issue due to the teratogenic effects observed in this study, and it is recommended that these effects be explored more extensively with variable doses in other animal species.

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