ARTICLE / INVESTIGACIÓN

Studies on teratogenic and maternal effects of Trichothecene (TCT) extracted from *Fusarium* and *Trichoderma* culture on pregnant Albino Mice

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Abstract: Mycotoxins (frequently referred to as secondary metabolites of toxigenic fungi) are commonly found in foodstuffs and are essential because of their association with disease. The current study investigated the teratogenic potential characteristics of T-2 trichothecenes mycotoxins produced by *Fusarium* and *Trichoderma*, which are reportedly toxic to humans and animals in pregnant mice. T-2 toxin is a cytotoxic secondary fungal metabolite that belongs to the trichothecene mycotoxin family. T2 was administered intraperitoneally to Pregnant females as a single injection at levels of 50, 75,100, and 125 ppb on one of the gestation days 7-11 during the pregnancy period. A significant reduction in mean fetal body weight and various fetal malformations (i.e., external and skeletal) were observed following maternal exposure to T2. This is the first report to implicate this mycotoxin T -2 as a teratogen in our region. Our study revealed that T-2 toxin 125 ppm exposure was enough to cause abortion in pregnant mice within several days.

Key words: T2 toxin, trichothecenes, mycotoxins, fungi, pregnant mice.

Introduction

People in poor nations, particularly in rural regions, rely on locally produced foods and confront challenges with food security and mycotoxin contamination, a severe food quality concern[1,2]. Chemical pollutants in food and feed provide a possible public health risk. In this regard, mycotoxins are among the most common contaminants in the food chain[3]. Depending on the ecological circumstances, fungi create mycotoxins in various agricultural products. Under specific temperature and humidity circumstances, multiple fungi, including *Penicillium, Aspergillus*, and *Fusarium*, release these poisons that deteriorate agrarian products. It is estimated that approximately 25% of the world's agricultural commodities are contaminated to some extent with mycotoxins[4,5]. Mycotoxins come in more than 400 different varieties, with fumonisin, ochratoxin, aflatoxin, zeranol, trichothecenes, and patulin. The most significant class of Fusarium mycotoxins, trichothecenes, also have the most varied chemical makeup. They are part of a comprehensive family of mycotoxins with numerous chemical similarities[6]. Trichothecenes can be produced by *Fusarium, Myrothecium, Spicellum, Verticimonosporium, Cylindrocarpon*, and *Stachybotrys*, even though these organisms belong to distinct taxonomic groups. Trichothecenes are one of the potential hazards to both human and animal health on a global scale[7]. Trichothecenes are widely distributed and have molecular weights between 200 and 500 Da. They contain over 200 poisons, with or without macrocyclic esters, ester ether bridges between C-4 and C-15, and a substantial sesquiterpenoid structure[8]. One of the more well-known I 2: I 3 epoxy trichothecenes are T-2 (Figure. I), and identifiers of T-2 and HT-2 are given below.

Moreover, it has been the subject of various toxicological research, most done after oral administration. The current study was, therefore, undertaken to provide further information on the feticidal and teratogenic effects of maternally injected trichothecene on albino mice in Duhok province.

Materials and methods

Mycotoxins obtained from the mycology research lab at the College of Science University of Duhok extracted from the culture of Fusarium and Trichoderma using the method of Bragulat, 2001 and determined by the ELISA method (T 2 vertex company).

Animals and Experimental Procedures

This study was held in the Animal House/College of Science/University of Duhok and approved by the Scientific Ethics Committee of the College of Science University of Zakho. The mice were fed regular food as well as water. Adult (6–8 weeks) female albino Swiss mice weighing 25–30 g were purchased. Animals were housed in individual cages measuring (50X50 cm), at animal house facilities with a controlled temperature (25 °C ± 1 °C) and with a 40 [±5] % relative humidity under a 12-h light-dark cycle with free access to a standard pellet diet and water. Virgin females were kept in separate cages and mated throughout one night with a male of the same strain when they were 60–70 days old and weighed 20–24 g. A vaginal obstruction was present the following morning, referred to as a good mating. The female mice were not treated and used as sires. Only pregnant females were separated and arbitrarily placed in either the treatment or control groups. The mice were acclimated to their environment for 1-week Pregnant albino mice of five

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groups. Group 1 received 50 ppb of T-2 during three weeks of pregnancy, G 2 received 75 ppb of T-2 during three weeks of pregnancy, G3 received 100 ppb of T-2 during three weeks of pregnancy, G4 received 125 ppb of T-2 during three weeks of pregnancy. G5 control female pregnant mice received 0.25 normal saline for 20 days. The mice were fed conventional pellets and distal water. They were tested for illness by administering systemic antibiotics to ensure they were healthy before the trial started.

Results

Mycotoxins are highly diverse secondary metabolites produced in nature by a wide variety of fungus which causes food contamination, resulting in mycotoxicosis in animals and humans. In particular, trichothecenes mycotoxin produced by Fusarium and Trichoderma is agriculturally more critical worldwide due to their potential health hazards. While several studies have demonstrated the toxicity and carcinogenicity of different food-contaminating mycotoxins, there is little information on the feticidal and teratogenic effects of maternal ingestion of these compounds. Furthermore, studies on the teratogenicity of trichothecene have yielded unambiguous findings[9].

Intrauterine growth, fetal mortality, and incidence of anomalies

Various types of malformations caused by trichothecene are shown in Table 1. Abnormalities caused by T2 differ from case to case and include exencephaly. The eyelids are open, the protection of intestines is short and hocked, the tail cleft pinna /open ear, and the jaw has skeletal abnormalities, including fused ribs and vertebrates.

The values for embryonic resorptions, late fetal deaths, and mean pup weights in Group 1, which received 50 ppb on embryonic development days 8 and 9, were similar to those of non-treated controls. (Group 5). The exposed fetus abnormalities on days 8 and 9 were not visible when the toxin was administered, as shown in Figure 2.

Both days of therapy with Trichothecene (Group II) they have revealed both teratogenic and foetotoxic effects. According to the day the toxin was administered, the incidence of gross malformations and late fetal deaths differed. Among 60 mice tests, the external anomalies exencephaly during gestation day 8 was 3 (5%) and 5 (8.3%) during day 9 of gestation regarding open eyelid during gestation day 8 was 1 (0.6%) and 2 (0.3%) during gestation day 9. Cleft pinna / open ear abnormally just found in gestation day 9 (0.6 %). Only during gestation day 9, 1 (0.6%) shows a Malformed jaw and lack of tail, while 2(0.3 %) and 9 shows a Short and hooked tail. Regarding skeletal anomalies, 2 (0.3) and during gestation day 9 show fused ribs, and 1 (0.6 %) in both days 8 and 9 show fused vertebrae. T2 readily passes the placenta and directly affects the fetus, resulting in an abnormality in skeletal muscle.

Group 3 of therapy with mycotoxin trichothecene also revealed both teratogenic and foetotoxic effects; among total mice tested the external anomalies exencephaly during gestation day 8 was 4 (2.4 %) and 8 (13 %) during day 9 of gestation .regarding open eyelid, Cleft pinna / open ear abnormally and Malformed jaw during gestation day 8 was 2 (1.2%) and 5 (3%) during gestation day 9 , 2 (1.2 %) during gestation day 8 and 9 shows Protesion of the intestine and the short and hooked tail rat was 2 (1.2 %) during gestation day 8 and 3 (1.8%) during gestation day 9. Regarding skeletal anomalies, 1 (0.6) during gestation day 8 and 3 (5%) shows fused ribs, and 1 (0.6 %) in both day 8 and 9 shows fused vertebrae. In addition to previous teratogenic effects, the fetuses of pregnant rats given 75 and 100 ppb of T-2 have various external malformation defects. Subcutaneous hematoma (red patches on multiple areas of the body) is exhibited in the following regions: (tail, face, back, belly, fore, and hind limbs) and clubfoot, open eyelid, and open mouth, as illustrated in Fig.3 as well as placental hemorrhage day 7 and 11 of gestation.

Discussion

Mycotoxins may cause morbidity due to positive organ pathology, even without lethal effects on test animals during experimentation. The extent of the mycotoxin hazard can be better assessed if the identity of the fungi involved, the affected organs, and the type of damage are known..T-2 toxin's harmful effects have been researched in experimental animals, including poultry, cattle, sheep, and pigs, all of which appear to be vulnerable to this mycotoxin. Pigs are the most sensitive farm animal species[10]. Due to microbial breakdown among rumen microorganisms, ruminants are more resistant to the adverse effects of T-2 toxin[11]. Animal experiments are the first scientific preference for determining whether a chemical is teratogenic and for estimating the potential teratogenic effect of any particular substance on human health. In the current study, the effect of trichothecene 2 was studied on albino male mice. T-2 toxicity and adverse effects vary depending on several parameters, including the mode of administration, the duration and quantity of exposure, the dosage delivered, and the animal's age, sex, and overall health, as well as the presence of any other mycotoxin[12]. A T-2 dose of 50 ppb has no significant effect on pregnant mice. There were no indicators of harm

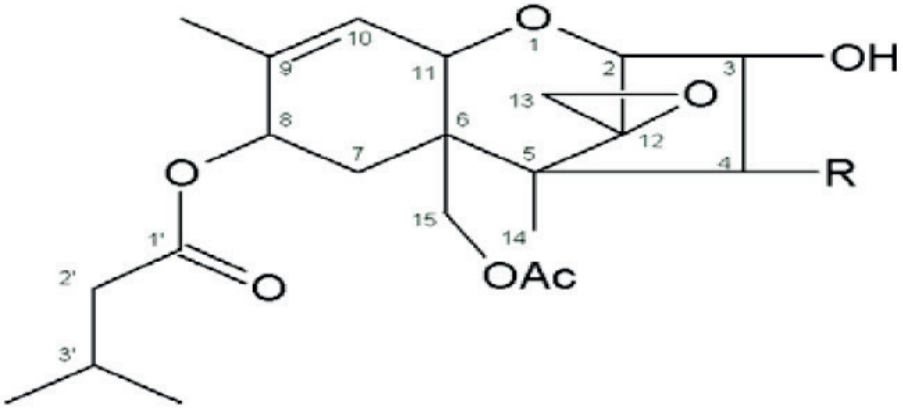

Figure 1. Structure of T-2 (R = OAc) and HT-2 (R = OH) toxin.

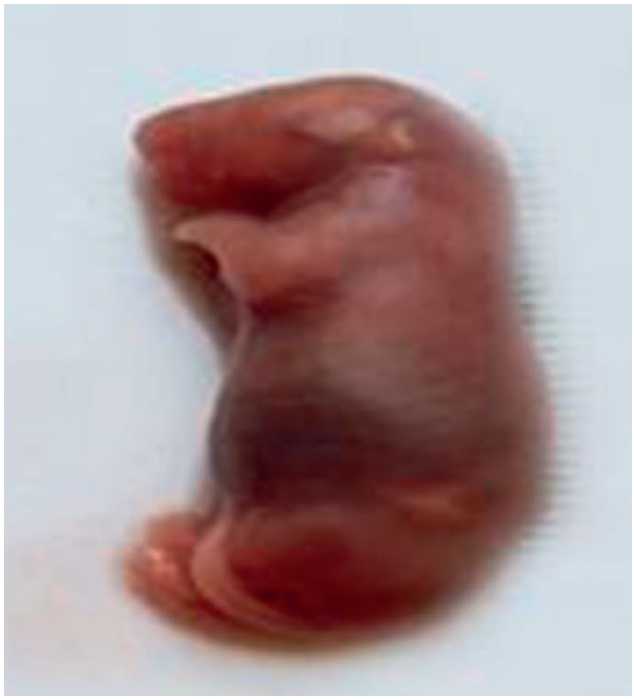

Figure 2. A photograph of an untreated control baby on the 21 days of pregnancy demonstrating normal morphology with average weight and length.

taining numerous proliferating component cells. The direct cytotoxic action of T-2 toxin on the fragile vasculature in the labyrinth zone may be the source of such placental bleeding[14,15], T-2 toxin influences the coagulation system, either by increasing or decreasing clotting factors[16]. It also displayed clubfoot, open eyelids, and an open mouth, indicating that the polyrelaxant medication directly influenced embryo development. These effects were shown by using different doses, including 75 ppb and 100 ppb. A daily record of the behavior (appetite, activity, and response to external stimuli) and general health of all of the animals was maintained. This study exhibited an increased incidence of skeletal abnormalities, summarized in Exencephaly Malformed jaw Provision of intestine Short and hooked tail Fused ribs and vertebrae. As seen in Table 1, the feticidal and teratogenic effects of T-2 occurred after treatment on either day (8-9)of pregnancy. All pregnant mice given 125 ppb caused abortion within the first week of pregnancy. The thiol group in T-2 toxin makes it a strong protein and DNA synthesis inhibitor. It also lowers lymphocyte proliferation, affects membrane function, hinders antibody generation, and modifies dendritic cell development[17,18].

Conclusions

Fungal secondary metabolites, such as the T-2 toxin, have caused severe side effects and continue to damage

Types of malformation		75ppb		100ppb	
External :	Gestation day	8	9	8	9
Exencephaly		3 (1.8%)	5 (8.3%)	4(2.4%)	8(4.8%)
Open eyelid		1 (0.6%)	2 (1.2%)	2 (1.2%)	5(3%)
Cleft pinna / open ear		0	1(0.6%)	2(1.2%)	5 (3%)
Malformed jaw		0	1(0.6%)	2(1.2%)	5 (3%)
Lack tail		0	1(0.6%)	2 (1.2%)	2 (1.2%)
Short and hooked tail		0	2 (1.2%)	2 (1.2%)	3(5%)
Skeletal					
Fused ribs		0	2 (1.2%)	1 (0.6%)	3(5%)
Fused vertebrae		1 (0.6%)	1(0.6%)	1 (0.6%)	1(0.6%)

Table 1. Frequency of various types of malformations associated with prenatal exposure to T2 (75ppb) and (100ppb) on Gestation day 8-9.

associated with T-2 in pregnant mice as compared to the control group during the gestation period, as evidenced by vaginal bleeding, abortion, or miscarriage. Our findings revealed certain morphological abnormalities that appeared as subcutaneous hematoma (red patches on various sections of the body) that might be attributed to trichothecene anticoagulant action in the following areas: (tail, face, back, abdomen, fore, and hind limbs)[13]. T-2 toxin easily crosses the placenta and is delivered to embryo/fetal tissues con-

agricultural animals worldwide. T-2 toxins and their metabolites occur in a variety of nations but are most common in tropical and subtropical climates, the current study has provided further evidence that mycotoxins present in the food chain affect not only the health of the mature individual but the unborn is also at risk and caused long-term transgenerational toxicity on the development and reproduction of female offspring.

Figure 3. A photograph of the treated baby on the third week of pregnancy demonstrating: A. different weight with a short tail B-hand defect C-Fetus with back hematoma and hooked tail.

Figure 4. A photograph of the treated baby on the third week of pregnancy demonstrating a lack of tail and short tail.

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