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Recreational use of marijuana during pregnancy and negative gestational and fetal outcomes: An experimental study in mice

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ABSTRACT

The prevalence of marijuana use among pregnant women is high. However, the effects on gestation and fetal development are not well known. Epidemiological and experimental studies present conflicting results because of the route of administration, dose, time of exposure, species used, and how *Cannabis* toxicity is tested (prepared extracts, specific components, or by pyrolysis). In this study, we experimentally investigated the effects of maternal inhalation of *Cannabis sativa* smoke representing as nearly as possible real world conditions of human marijuana use. Pregnant mice (n = 20) were exposed (nose-only) daily for 5 min to marijuana smoke (0.2 g of *Cannabis*) from gestational day (GD) 5.5 to GD17.5 or filtered air. Food intake and maternal weight gain were recorded. Ultrasound biomicroscopy was performed on 10.5 and 16.5dpc.On GD18.5, half of the dams were euthanized for the evaluation of term fetus, placenta, and resorptions. Gestation length, parturition, and neonatal outcomes were evaluated in the other half. Five minutes of daily (low dose) exposure during pregnancy resulted in reduced birthweight, and litter size was not altered; however, the number of male pups per litter was higher. Besides, placental wet weight was increased and fetal to placental weight ratio was decreased in male fetuses, showing a sex-specific effect. At the end of gestation, females from the *Cannabis* group presented reduced maternal net body weight gain, despite a slight increase in their daily food intake compared to the control group. In conclusion, our results indicate that smoking marijuana during pregnancy even at low doses can be embryotoxic and fetotoxic.

1. Introduction

Cannabis sativa is popularly known as marijuana and smoking is the commonly used form of this drug. Based on unofficial estimates of drug consumption conducted by the United Nations, it is the most abused drug in the world, with 140 million consumers (UNODC, 2015). Users are young, and exposures occur during their reproductive age (SAMSHA, 2015). Moreover, among pregnant women, it appears more frequently in self-reported questionnaires of drug use during gestation (ACOG, 2015; SAMSHA, 2010). Most of the studies on the toxicity of marijuana use during pregnancy have evaluated the neuro-behavioral effects (Higuera-Matas et al., 2015; Campolongo et al., 2011).

Epidemiological evidence has shown that marijuana impairs the growth trajectory of the fetus, resulting in low birth weight, intrauterine growth retardation (IUGR) and congenital malformation (Hurd et al., 2005; El Marroun et al., 2011; Sherwood et al., 1999; Zuckerman et al., 1989; Fried et al., 1984; Gibson et al., 1983). Maternal health is also negatively affected; marijuana-using mothers present higher prevalence of dysfunctional and precipitous labor, as well as meconium-stained amniotic fluid (Alharbi and El-Guebaly, 2014). Other potential adverse effects of smoking marijuana during pregnancy are lesser known.

The majority of the toxicological knowledge about the effects of *Cannabis sativa* on the reproductive tract and fetal development comes from animal studies. In these studies, exposures are done primarily by gavage of marijuana extract or Δ^9-THC i.p (Abel, 1975). Regardless of the route of administration (inhalation, i.p., p.o., i.v.,

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and i.p), findings of these studies demonstrate increased resorption rate and reduced fetal weight in both mice and rats (Rosenkrantz et al., 1978; Persaud and Ellington, 1967; Kostellow et al., 1978; Harbison and Mantilla-Plata, 1972; Joneja, 1976; Fleischman et al., 1980). None of the studies have reported fetal malformations. Abel et al. (1981) reported that pregnant rats exposed to different doses of *Cannabis* extract (20, 200 mg/kg) throughout gestation presented reductions in weight gain and food consumption. Birth weight was reduced only in those groups exposed during the third week or during the whole gestation. Charlebois and Fried (1980) evaluated the effects of pre-gestational and gestational exposure to *Cannabis* smoke on rats fed low, normal, or high protein diet. They observed that *Cannabis* exposure lengthened the gestation period and increased the occurrence of stillbirths and litter destruction. When exposure was coupled with a high protein diet, these effects were attenuated. Furthermore, evaluation of the outcomes of the groups exposed both before and during gestation suggested a degree of tolerance to the drug effects.

THC and its metabolites are able to cross the placental barrier and reach the fetus (Hutchings et al., 1989; Jakubovic et al., 1973). The endocannabinoid (eCB) system has an important role in reproduction, from the earliest stages of ontogenic development to parturition, including fertilization, embryo implantation, and placentation (Sun and Dey, 2012). The endocannabinoid system is present in different organs where it plays multiple physiological roles. It is composed of the cannabinoid receptors, CB1 and CB2, which are G protein-coupled receptors that are differentially distributed in the organs (Park et al., 2003; Das et al., 1995; Galiegue et al., 1995), and endogenous molecules (endocannabinoids) derived from arachidonic acid: anandamide (*N*-arachidonylethanolamine—AEA) and 2-arachidonoylglycerol (2-AG). Marijuana's Δ^9-THC can also bind to CB receptors and activate multiple intracellular signal transduction pathways.

Studies in humans have many confounding factors (e.g., lifestyle, socioeconomic and nutritional status, age, and tobacco use) that make it difficult for interpretation and establishment of a causal relationship between smoking marijuana and poor gestational outcomes. Furthermore, toxicological studies conducted in animals use intraperitoneal injections or oral gavage of Δ^9-THC to perform the exposures, which exclude the interaction of compounds present in the smoke that could also contribute to pregnancy disorders, and the doses used are far beyond the dose commonly experienced by humans (Abel, 1975). Most of the published reviews have acknowledged that there are several uncertainties on the effects of maternal marijuana use on gestational and fetal outcomes (Volkow et al., 2014). There is lack of information on biological mechanisms, whether fetal developmental disruptions occur indirectly (maternally mediated), directly, or as a combination of both, and alterations in placental function, changes in hormonal balance, on sex-specific effects, effects on organogenesis of the kidney, lungs, spleen, and thymus.

These aspects and the spreading legalization of recreational use of *Cannabis sativa* point out that there is an urgent need of further toxicological studies to better recognize the effects and elucidate the mechanism involved in this association. In the present study, we developed an experimental murine model to study the effects of recreational use of marijuana during pregnancy to mimic human "real world" exposures in terms of dose and use to evaluate the effects on gestational and fetal outcomes.

2. Material and methods

2.1. Animals

This study was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science of the University of São Paulo (Protocol no. 5910070714). We conducted the experiments in agreement with the National and Institutional Guidelines for Animal Welfare. All animals were treated humanely with due consideration being given to the alleviation of distress and discomfort. Two groups of Balb/C mice (inbred strain) were used in this study: 20 females aged 60 days and 3 males aged 80 days, with proven fertility from our University's Animal Facility. We raised and maintained the animals in a ventilated cage system with food and water *ad libitum*. Temperature ($21 \pm 2\,^{\circ}C$) and light (12/12-h light/dark cycle) were controlled.

2.2. Drug

Marijuana (containing 0.3% Δ^9-THC) used in this study was donated for scientific purposes by the Núcleo de Perícias Médica Legais—Instituto de Criminalística de Marília legally authorized by the 3° Vara Criminal da Comarca de Marília, São Paulo, Brazil. The drug comes from a drug bust conducted by the local police.

2.3. Exposure system

The exposure system (Fig. 1) is composed of a pump that blows air through a HEPA filter into a pulse dumper. The airflow is split into two directions that pass across the valves that control the flow. One flow goes to the smoking chamber and the other directly to the mixture chamber. The smoking chamber is a 1-L sealed box with an aperture for the airflow, creating a positive pressure that forces the airflow to pass through the cigarette, and consequently the smoke flows to the mixture chamber. The mixture chamber is a 1-L sealed box with a bulkhead to promote the mixing process. Air-smoke flow was controlled at 1.2 L/min. The mixed smoke-air goes to the manifold where mice are arranged in individual tube-type holders (n = 8). The prepared marijuana cigarette lasts for 5 min of exposure in this system. An identical system was built to conduct exposures to the control group.

2.3.1. Marijuana cigarettes

Marijuana cigarettes were prepared by grinding 200 mg of *Cannabis sativa* and manually filling commercially available blank cigarette tubes. This allowed us to prepared standardized cigarettes.

2.4. Exposure protocol

Twenty healthy female mice were randomly distributed in the *Cannabis* or control group (n = 10 mice per group). During 7 consecutive days, all females were trained to be familiar with the experimental procedures and researchers.

After the training period, the females were housed with males (2:1), and the presence of a vaginal plug or sperm in the vaginal lavage was considered as the evidence of mating and the 0.5 gestational day (GD) was determined. Pregnant mice were exposed daily for 5 min to marijuana smoke or filtered air from GD 5.5–17.5 (Fig. 2). The exposure time was similar to that described by Lichtman et al. (2001), with some modifications.

2.5. Characterization of the exposure

Maternal exposure was characterized by the presence of Δ^9-THC metabolites (THC–COOH) in urine samples. Urine was collected every 24 h after exposure, according to the protocol of Khosho et al. (1985).

2.5.1. Chemicals

The following chemicals reagents were used: sodium hydroxide (Merck—Darmstadt, Germany UK), acetic acid (Merck—Darmstadt,