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ALTERAÇÕES COMPORTAMENTAIS E DE DESENVOLVIMENTO ASSOCIADAS A DROGAS PSICOATIVAS EM HUMANOS E MODELO DE *DROSOPHILA MELANOGASTER*

Maceió
2023

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Tese de doutorado apresentada ao Programa de Pós-graduação em Ciências da Saúde da Universidade Federal de Alagoas, como requisito parcial à obtenção do título de Doutora em Ciências da Saúde.

Orientador: Prof. Dr. Olagide Wagner de Castro.

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“Todo o progresso é precário, e a solução para um problema coloca-nos diante de outro problema.” (Martin Luther King)

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LISTA DE ABREVIATURAS E SIGLAS

AEME - Éster Metílico de Anidroecgonina

CNS - central nervous system

DA – Dopamine

DopR - DA receptor mutant flies

DVMAT - Isoform of Vesicular monoamine transporters in Drosophila

ET50 - Number of days required for 50% of flies to hatch

HC - head circumference

HSM - Hempseed meal

ISTs - Infecções Sexualmente Transmissíveis

LMO - LIM-domain-only proteins

OXT - oxytocin

PKA - Protein kinase A

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-analyzes

PROSPERO - International Prospective Register of Systematic Reviews

ST50 - Time to 50% sedation

SOD - Superoxide dismutase

THC - Tetrahydrocannabinol

TNT - Tetanus toxin

VMATs - Vesicular monoamine transporters

5HT - Serotonin

SUMÁRIO

APRESENTAÇÃO

O presente trabalho foi desenvolvido para contribuir com conhecimento sobre os efeitos das drogas de abuso. O trabalho foi motivado inicialmente a partir de pesquisas clinicas desenvolvidas no nosso laboratório nas quais surgiram diversos questionamentos. A construção desse trabalho auxiliou no desenvolvimento dessas pesquisas e acreditamos que contribuirá para incrementar em futuros trabalhos da área.

O conteúdo do trabalho foi dividido em dois artigos de revisão sistemática, o primeiro aborda as consequências maternais, fetais e neonatais relacionadas ao uso de crack durante o período gestacional. É uma revisão realizada a partir de estudos observacionais. O segundo artigo aborda as alterações encontradas no modelo de *Drosophila melanogaster* quando submetida ao tabaco, maconha ou cocaína.

RESUMO - ARTIGO 1

O consumo de crack é um dos principais desafios de saúde pública com um número crescente de crianças intoxicadas por crack durante o período gestacional. O objetivo principal deste trabalho foi avaliar os achados acumulados e fornecer uma perspectiva atualizada sobre este campo de pesquisa. Uma revisão sistemática com metanálise foi realizada usando o modelo de efeitos aleatórios, razão de chances (odds ratio) para variáveis categóricas e diferença média para variáveis contínuas. A heterogeneidade estatística foi avaliada usando a estatística I-quadrado e o risco de viés foi avaliado por meio da Escala de Avaliação de Qualidade de Newcastle–Ottawa. Dez estudos preencheram os critérios de elegibilidade e foram usados para extração de dados. O uso de crack durante a gravidez foi associado a chances significativamente maiores de parto prematuro (OR, 2,22; IC 95% , 1,59–3,10), descolamento prematuro de placenta (OR, 2,03; IC 95% 1,66–2,48), menor perímetro cefálico (−1,65 cm; IC 95% −3,12 a −0,19), PIG (pequeno para a idade gestacional) (OR, 4,00; IC 95% 1,74–9,18) e baixo peso ao nascer (BPN) (OR, 2,80; IC 95% 2,39–3,27). Esta análise forneceu evidências claras de que o crack contribui para resultados perinatais adversos. A exposição de crack no período pré-natal está associada ao BPN, parto prematuro, descolamento placentário e menor circunferência de cabeça.

Palavras-chave: Peso ao Nascer. ·Crack. · Idade Gestacional. · Gravidez. · Prematuridade

1 INTRODUÇÃO

As drogas psicoativas são substâncias que, quando em contato com o organismo, interagem com o sistema nervoso central afetando os processos mentais, como a percepção, consciência, cognição ou humor e emoções (WORLD HEALTH ORGANIZATION, 2022). Entre as substâncias mais consumidas estão a maconha, tabaco, cocaína e crack (BASTOS, 2017; INCA, 2022). De acordo com as últimas estimativas, há aproximadamente 20 milhões de usuários de cocaína em todo o mundo (UNITED NATIONS OFFICE ON DRUGS AND CRIME, 2021). No Brasil, 3,9% da população já consumiu cocaína pelo menos uma vez na vida e cerca de 1,5% já usou crack (ABDALLA et al., 2014). As mulheres representam 21,3% dos usuários de crack em áreas de uso de drogas (MEDEIROS; MACIEL; SOUSA, 2017).

A cocaína é extraída das folhas da planta de coca. A substância é refinada em uma pasta e depois processada em uma substância em pó branca. A substância em pó é mais frequentemente aspirada, misturada com um líquido e injetada ou fumada. Já o crack, é essencialmente a mesma substância que a cocaína, porém em uma forma sólida (pedra), o que o torna mais passível de ser fumado. O processo de fabricação do crack envolve misturar a forma em pó da cocaína com alguma base, como bicarbonato de sódio ou outra substância, fervê-la em água e, em seguida, remover o bicarbonato de sódio (HATSUKAMI; FISCHMAN, 1996).

A substância éster metílico de anidroecgonina (AEME) é um composto exclusivamente gerado na pirólise do crack e tem sido associado ao maior efeito neurotóxico quando comparado a cocaína (GOMES et al., 2018). Além disso, durante a gravidez, essa droga atravessa facilmente a placenta (BELL; LAU, 1995; GANAPATHY, 2011) contribuindo para um fenômeno importante: o aumento do número de crianças (crack babies) intoxicadas por *crack* durante a gestação (DUAILIBI; RIBEIRO; LARANJEIRA, 2008). O uso de *crack* na gravidez acarreta em prematuridade, malformação congênita, internação em unidade de terapia intensiva, uso de cuidados e tecnologias de alimentação por meio de fórmulas lácteas artificiais (MODERNEL XAVIER et al., 2017). Uma metanálise do nosso grupo (parte deste trabalho de doutorado) realizada a partir de estudos observacionais em humanos, identificou que a exposição materna ao crack está relacionada ao baixo peso ao nascer, parto prematuro, descolamento prematuro de placenta e menor perímetro cefálico (DOS SANTOS et al., 2018). Dados do nosso grupo, demonstram ainda que o uso de crack no período gestacional leva a comportamentos depressivos e ansiosos, comprometimento da memória e aumento da

suscetibilidade a convulsões na prole de ratos (PACHECO et al., 2021).

A literatura sugere que o uso de crack é fator que contribui para a troca de sexo por drogas ou dinheiro aumentando os riscos para as Infecções Sexualmente Transmissíveis (ISTs) entre as mulheres (LOGAN; LEUKEFELD; FARABEE, 1998). Além disso, o uso de crack entre mulheres em idade reprodutiva aumentou em poucos anos (BLAYAC et al., 2022), incluindo o poliuso de drogas (ENGLAND et al., 2020). O abuso de drogas durante a gravidez pode afetar a saúde da mulher, interferindo em sua capacidade de cuidar de si e, portanto, prejudicando a realização do pré-natal (BOTELHO; ROCHA; MELO, 2013; NARKOWICZ et al., 2013; SILVA et al., 2013). E as mulheres usuárias de drogas ainda são percebidas pela população e pelos profissionais de saúde como irresponsáveis, criminosas e profissionais do sexo (CRUZ et al., 2014). Dessa forma, vários fatores contribuem para desfechos negativos para a mãe e ou o feto.

Nesse contexto, o nosso objetivo foi descrever, a partir de dados elencados na literatura, as consequências maternais, fetais e neonatais associadas ao uso do crack durante o período gestacional. Este estudo foi publicado na “Archives of Gynecology and Obstetrics”.

Artigo na íntegra

ARTIGO 1

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Maternal, fetal and neonatal consequences associated with the use of crack cocaine during the gestational period: a systematic review and meta-analysis

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Abstract

Objective. Crack cocaine consumption is one of the main public health challenges with a growing number of children intoxicated by crack cocaine during the gestational period. The primary goal is to evaluate the accumulating findings and to provide an updated perspective on this field of research. **Methods**. Meta-analyses were performed using the random effects model, odds ratio (OR) for categorical variables and mean difference for continuous variables. Statistical heterogeneity was assessed using the I-squared statistic and the risk of bias was assessed using the Newcastle–Ottawa Quality Assessment Scale. Ten studies met eligibility criteria and were used for data extraction. **Results.** The crack cocaine use during pregnancy was associated with significantly higher odds of preterm delivery (OR, 2.22; 95% CI, 1.59–3.10), placental displacement (OR, 2.03; 95% CI 1.66–2.48), reduced head circumference (−1.65 cm; 95% CI −3.12 to −0.19), small for gestational age (SGA) (OR, 4.00; 95% CI 1.74–9.18) and low birth weight (LBW) (OR, 2.80; 95% CI 2.39–3.27). **Conclusion**. This analysis provides clear evidence that crack cocaine contributes to adverse perinatal outcomes. The exposure of maternal or prenatal crack cocaine is pointedly linked to LBW, preterm delivery, placental displacement and smaller head circumference.

Keywords. Birth weight. Crack cocaine. Gestational age. Pregnancy. Prematurity

1 INTRODUCTION

The consumption of psychotropic substances has been increased substantially with the development of new forms of use and compositions. Crack cocaine is a smoked form of cocaine which has several psychotropic and neurotoxic effects, potentialized by products generated from cocaine pyrolysis [1]. Its consumption leads to short-duration euphoria as a consequence of high bioavailability and rapid metabolism, followed by intense craving [2]. In consequence, crack cocaine users are inclined to violent behavior and promiscuity to acquire the drug, increasing the risk of sexually transmitted disease's contamination [3–6]. Of particular interest is the consumption of cocaine and derivatives during pregnancy, which has lead to an increasing number of children intoxicated by crack, identified in the literature as "crack babies" [7]. In fact, cocaine and crack cocaine cross the placental barrier [8, 9] and promote prolonged effects on the pregnant woman, as well as the embryo or fetus. The net result is aggravated because, in the gestational period, the drug metabolism is delayed due to decreased expression of plasma and hepatic cholinesterases [10]. Aghamohammadi and Zafari [11] For instance, pregnancies in crack users are associated with higher incidence of fetuses with intrauterine growth restriction, preterm births, placental abruption, and preeclampsia. Children born to crack users exhibit cognitive deficits, difficulty in verbalization, aggressiveness as well as depression [12]. Although the biological mechanisms underlying these alterations are unclear, there are studies which suggest that crack cocaine acts on both the maternal and fetus central nervous system (CNS), inhibiting dopamine, noradrenaline and serotonin reuptake at the presynaptic terminals, and exacerbating their effects on the effector organs [8, 13]. In the fetus, the adrenergic effects may lead to reduced placental flow, which in turn, can negatively impact fetal growth and oxygenation [14]. Newborns of crack users may not respond organically to environmental stimuli, in addition to commonly presenting fever, irritability, sweating, seizures, and vomiting, which may be associated with changes in brain content of dopamine and serotonin, typifying a withdrawal syndrome [8, 14, 15]. The effects of cocaine derivatives on the development of the fetus, children, adolescents, and adults are serious public health problems [16]. Furthermore, despite the growing number of publications, translational research focused on clinical treatment in this field is limited. In this systematic review, we provide a critical appraisal of the current information and evaluate by meta-analysis the findings on maternal, fetal and neonatal consequences caused by crack cocaine exposure during pregnancy.

2 METHODS

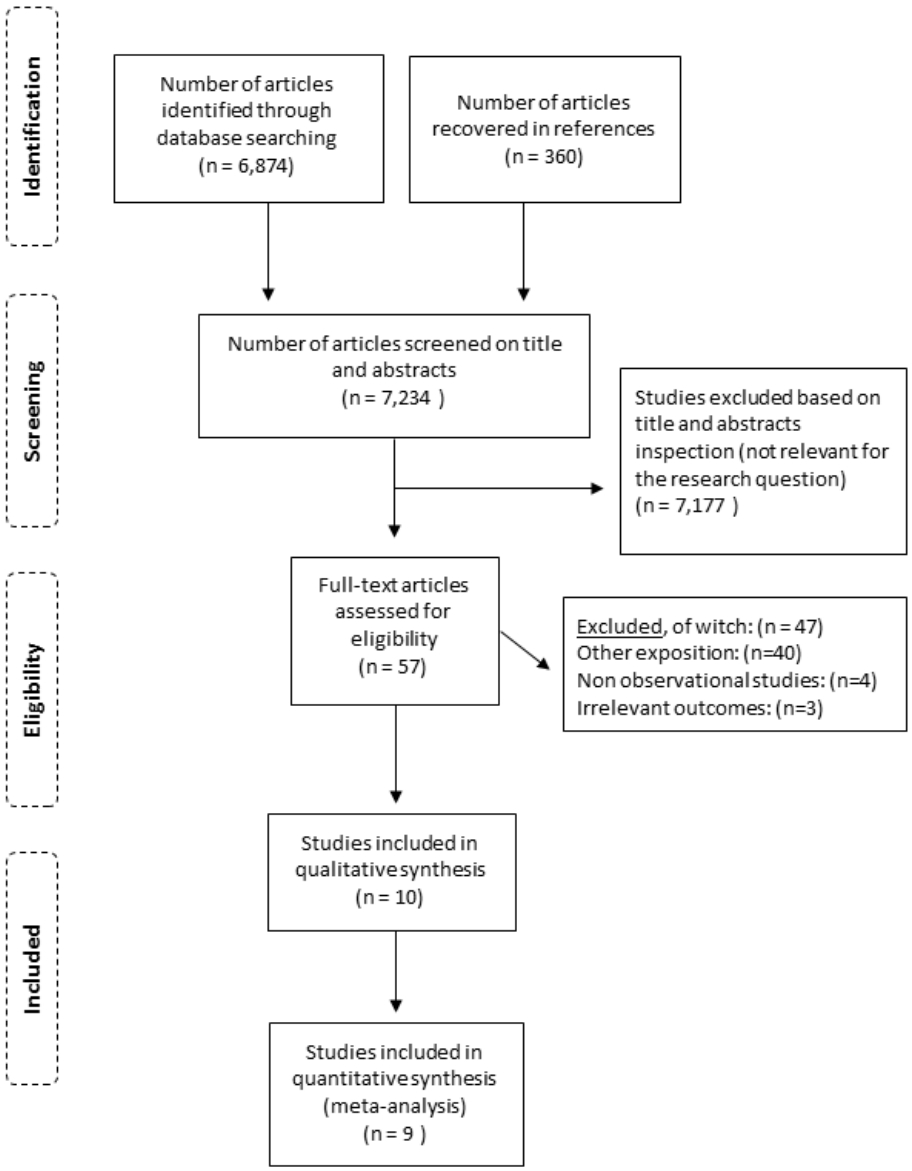
The study was performed following the guidelines of the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA Statement) [17]. The execution was developed in accordance with the recommendations of the Moose Group (MOOSE Guidelines) [18]. The protocols were registered on International Prospective Register of Systematic Reviews (PROSPERO; nº. CRD42017052277), and it is available on https://www.crd.york.ac.uk/PROSPERO/ display_record.asp?ID=CRD42017052277. The search strategy aimed to identify observational (retrospective and prospective) and case-control studies evaluating the maternal, fetal and neonatal consequences of crack cocaine exposure during the gestational period. Articles published from the starting date of each database to January 2017 were included. No language restriction was applied. Databases searched included PubMed (from 1960 to January 2017), EMBASE (from 1947 to January 2017), LILACS (from 1982 to January 2017), CINAHL (from 1984 to January 2017), Science Direct (from 1997 to January 2017), Google Scholar (from 2004 to January 2017), SIGLE (from 2007 to January 2017), Greylit (from 1999 to 2016) and ISI Web of Science (from 1900 to January 2017). The search procedure was performed from 16 January to 01 March 2017. A search strategy was performed using a combination of "pregnancy" and "street drugs" or "drugs of abuse" keywords (MeSH) was used. We did not use filters. The search strategies are shown in Table 1. In the first stage, the searches were conducted by two independent investigators, and their results were compared. Two reviewers (J.F.S. and C.M.B.C.) independently read all titles and abstracts. At a second stage, the reviewers read full-length manuscripts and reached consensus about their inclusion. The articles that met all the established criteria were included. The criteria for inclusion of articles required that pregnant women exposed to crack cocaine during pregnancy, and fetus or newborn was delivered by exposed mothers. Polydrug usage was not considered as an exclusion criterion since abuse of several drugs is common in cocaine users. We considered the control group as subjects which were not exposed to crack cocaine. Only original research from observational studies (cohort and case–control) was included. Comments, letters, editorials, and reviews were excluded. The references of included articles were checked for further eligible studies.

Table 1. Search strategies for each Database

PubMed	(("street drugs"[MeSH Terms] OR ("street"[All Fields] AND "drugs"[All Fields]) OR "street drugs"[All Fields] OR ("drugs"[All Fields] AND "abuse"[All Fields]) OR "drugs of abuse"[All Fields]) AND ("pregnancy"[MeSH Terms] OR "pregnancy"[All Fields]) NOT (("animals"[MeSH Terms:noexp] OR animal[All Fields]) AND study[All Fields]))
EMBASE	'crack cocaine'/exp OR 'drugs of abuse'/exp OR 'street drugs'/exp AND 'pregnancy'/exp
LILACS	Street drugs (pregnancy)
CINAHL	(MH "Crack cocaine+") OR (MH "street drugs+") AND (MH "Pregnancy+")
ScienceDirect	((pregnancy) and ("crack cocaine") and not ("animal study"))
Google Scholar	pregnancy “crack cocaine”
SIGLE	drugs of abuse
Greylit	street drugs
ISI Web of Science	(("street drugs") or ("drugs of abuse") AND (PREGNANCY))

The same reviewers conducted the initial data extraction independently. Studies excluded were recorded, and the reasons for exclusion are indicated in Fig. 1. Disagreements between reviewers were discussed in consensus meetings. General information from the studies was collected, such as year of publication, authors, geographic region of research, objective, study design, setting, participant sample, inclusion and exclusion criteria, data collection, data analysis, primary results, and author’s conclusions.

Fig. 1 Literature search flowchart



From each included paper, predetermined lists of variables were extracted. The primary outcomes of interest were maternal mortality, fetal mortality, intrauterine growth restriction, placental displacement, preeclampsia and congenital malformation. The secondary outcomes were low birth weight (<2500 g, very low birth weight 1500 g), preterm delivery (before 37 weeks), small for gestational age, head circumference, maternal and fetal length of hospital stay, and neonatal intensive care unit. We did not find studies that investigated the effect of maternal exposure of crack cocaine on maternal mortality, maternal and fetal length of hospital stay and

neonatal intensive care unit.

Risk of bias was assessed using the 9-point Newcastle–Ottawa Scale (NOS). This scale used a ‘star system’ in which a study is judged on three broad perspectives. These include the selection of the study groups, the comparability of the groups, and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies [19]. Studies were considered as a low risk of bias if they received seven or more stars, moderate if they received 4–6 stars, and high if they received up to three stars in the NOS quality assessment [20].

We provided a meta-analysis conducted using random effects modeling, odds ratio (OR) for categorical variables and mean difference for continuous variables. The Mantel–Haenszel test with a 95% confidence interval was applied. Statistical analysis was performed using STATA 13 software for meta-regression. However, for main analysis, Review Manager fve (RevMan fve) was used. Statistical heterogeneity was assessed using the I-squared statistic. Statistical heterogeneity was considered as significant if I-squared was more than 50%. Sensitivity analysis considered objective versus self-reported studies, retrospective versus prospective studies, and studies with a minimal or low risk of bias vis-à-vis studies identified as having a moderate or high risk of bias. Studies were considered as a low risk of bias if they received seven stars or more in the NOS quality assessment. If more than ten studies are included in the meta-analysis, a sensitivity analysis was conducted using meta-regression. A forest plot was used to demonstrate our results. Begg’s funnel plot and Egger’s test were not performed due to insufficient studies for meta-analysis.

3 RESULTS

Our search returned 7234 studies, being 6874 identified in database search and 360 recovered in references. 57 full-text articles were assessed for eligibility, and 47 were excluded. Among the reasons for the exclusion were other exposures (n=40, e.g., cocaine, amphetamine, methadone, tobacco and alcohol). Moreover, many studies were not observational (n=4, e.g., case reports, reviews or commentaries) and other studies had outcomes that were not of our interest (n=3, e.g., neurocognitive and behavioral effects). Ten studies met inclusion criteria and were included in this systematic review; however, one study was not included in the meta-analysis (Fig. 1).

Characteristics of the ten included articles are shown in Table 2. All of them were published in English between 1988 and 2016, being nine cohort studies and one case-control study. The exposure was mostly based on cocaine in its smoked form as crack cocaine and the effects were mainly ascertained through urine or meconium analysis and/or by chart review and/or maternal interview (objective report). In three studies, the exposure was evaluated by maternal self report. The outcome was mainly confirmed using physician examination or record examination.

Table 2. CHARACTERISTICS OF INCLUDED STUDIES

Author	Year of study publication/Duration of the study	Type of study	Setting of study	Population	Exposure assessment (How, when)	Outcomes assessed	Confounders adjusted for	Newcastle-Ottawa Scale Total of stars	Mainly Results	Remarks
Cherukuri et al	1988 / jul 1986 – nov 1986	Retrospective cohort with matched controls (Maternal Age, parity, PNC, SES, race, alcohol)	Kings County Hospital, New York, USA.	Patient delivering at Kings County Hospital, on public assistance	Maternal self-report at delivery	Head circumference, LBW, PTD, SGA, BW, GA		7	Intrauterine Growth retardation: crack exposed infants were 3.6 times more; Head Circumferenc e: crack exposed infants were 2.8 times more	
Chouteau et al	1988 / jan – dec 1986	Retrospective cohort with unmatched controls	St. Lukes's/Roosevel t hospital Center, New York, USA.	All pregnant woman admitted to the hospital Center in labor, who had not received prenatal care	Maternal objective-report and Maternal urine test at the time of admission	LBW, PTD		8	LBW: 43% for crack cocaine vs 23% for no users. PTD: 38% for crack cocaine vs 19% for no users.	were used Information about drug use in the charts, which showed that cocaine was most commonly smoked in freebase form as crack.
Petitti et al	1990 / jan 1987 – dec 1987	Case-control study	Alameda County, California, USA.	Eligible were singleton	Maternal objective-report in	< 1500 grams (very low birth weight),	Women were separately queried about	8	were estimated the relative risk of	Women were interviewed by

				infants without congenital anomalies whose mother and father were both Black or both White, non-Hispanic,	their homes.	1501-2500 grams and <37 menstrual weeks of gestation (low birth weight, pre-term), and 1501-2500 grams and -37 menstrual weeks of gestation (low birth weight, term, intrauterine growth retarded)	use of cocaine and of "crack" or freebase cocaine, which is the smoking of pure crystalline cocaine. Information about other potential confounders was also collected by interview.		low birth weight in relation to use of cocaine	interviewers that were trained
Bateman et al	1993 / sep 1985 – aug 1986	Prospective cohort with unmatched controls	Harlem Hospital in New york, USA.	population consisted of all mothers and their live-born singleton infants delivered at Harlem Hospital	Maternal self-report or infant urine	GA, BW, lenght, Head Circunference, LBW, PTD, adimitted neonatal to intensive care	Gestational age, maternal age, gravidity, race, Black/non-Black], sex of infant, receipt of prenatal care, maternal positive syphilis serology, and use of tobacco, alcohol, marijuana, and phencyclidine	9	LBW: Cocaine 30% vs unexposed 10%; Preterm birth: Cocaine 32% vs unexposed 14%; Birthweight: Cocaine 2713 ∓ 569 vs unexposed 3174 ∓ 573; Head Circumferenc e: Cocaine 32.5 ∓ 2.24 vs unexposed 34.0 ∓ 1.81	A group of 133 women who provided a strong history of crack cocaine use was identifiable.
Eyler et al	1994 / 1987 - 1988	Prospective cohort with matched controls (race, age, parity,	Regional tertiary medical center, Florida, USA.	all women receiving prenatal care at the local health	Maternal objective-report or screen urine mothers or	Abruptio placentae, Premature labor, Gestational		9	Congenital anomalies 7.7% vs 5.4%; Abruptio placenta 1.8%	The identification of prenatal crack cocaine use was

		prenatal care, alcohol and nicotine use)		department deliver. There are also some private patients and a few women presenting at the emergency room in active labor who deliver at the hospital.	infants. At the time of delivery.	age -< 37 weeks, Birthweight < 2500 g, Birthweight < 1500g, minute Apgar < 7, Resuscitation at delivery, Infant remained in nursery or intensive care unit beyond the mother's discharge, Congenital anomalies, and Perinatal deaths			vs 0.6%; Gestational age < 37 weeks 43.4% vs 31.5%; Birthweight < 2500 g 28.0% vs 17.9%; Birthweight < 1500g 8.9% vs 4.8%; 5-min Apgars < 7 16% vs 9.5%; Resuscitation 33.9% vs 18.4%; Remained in nursery 32.1% vs 16.1%; Fetal/neonatal death 1.8% vs 4.2% Gestational (week) 36.6 ± 5.0 vs 37.8 ± 3.41; Birthweight (g) 2704 ± 742 vs 2988 ± 7211; Apgar score at 5 min 8.3 ± 2.01 vs 8.5 ± 1.9.	determined by recorded history in the mother's chart. social workers report that of the women admitting cocaine use, 99% report they smoke crack.
Sprauve et al	1997 / jan – dec 1992	Retrospective cohort	Grady Memorial Hospital, located in Atlanta, Georgia	Inner-city, indigent, routine voluntary urine drug screening	Maternal objective-report at peripartum and Maternal urine at any time during pregnancy or within 1 wk	LBW, Fetal Growth Restriction, preterm delivery, abruptio placentae, low 5-minute Apgar scores, perinatal	multiple logistic regression was used to control for potentially confounding variables for alcohol,	9	LBW: users 31.3% vs non-users 14.9%; Growth-restricted infants: Users 29.0% vs non-users 13.0%;	

					of delivery	mortality, congenital malformations, and cesarean delivery rate.	tobacco, weight, age, PNC, PTB		preterm infants: Users 28.2% vs non-users 17.1%; abruptions: Users 3.3% vs non-users 1.1%;	
Eyler et al	1998	prospective, longitudinal cohort	Regional tertiary medical center, Florida, USA.	Women contacted the health care system, either at a prenatal obstetric clinic or the hospital.	Maternal objective-report at the end of each trimester and drug screening of urine specimens was required at the day consent was obtained and the day the infant was born.	LBW, PTD, fetal mortality, BW, GA, head circumference, SGA,		9	Compared with controls, the cocaine users had significantly higher Hobel Prenatal and Total Risk Scores and more preterm infants (28 vs 14), but not a significantly greater number of fetal deaths (3 vs 1	Interviewers were trained.
Richardson et al	1999 / marc 1988 – jun 1993	prospective, longitudinal cohort with unmatched contols	PC clinic at Magee-Womens Hospital, Pittsburgh, Pennsylvania	Women attending the PC clinic at Magee-Womens	Maternal objective-report antenatally	GA (wk), Weight (g), Length (cm), Head circumference (cm), LBW, SGA, Premature	PNC	8	In both samples, cocaine/crack use during early pregnancy predicted reduced gestational age, birth weight, length, and	

									head circumference , after controlling for the significant covariates of cocaine use. In a comparison of the samples, the offspring of the NPC/cocaine group were significantly smaller than were the offspring of the PC/no cocaine group, whereas the offspring of the PC/cocaine and NPC/cocaine groups did not differ.	
Behnke et al	2001 / 1991 - 93	prospective, longitudinal cohort with controls matched on race, parity, location of prenatal care	Regional tertiary medical center, Florida, USA.	contacted the health care system, at either a prenatal clinic or the hospital in the case of	Maternal objective-report at the end of each trimester and drug screening of urine	Birth weight, Length, Head circumference, Anterior fontanel, Posterior fontanel, Ear length Left,	Sufficient sample size was enrolled to allow for covariate analyses of	9	There were significantly more premature infants in the cocaine	if prenatal interviews were not possible because of limited or no prenatal care, women were

		(that related to level of pregnancy risk), and socioeconomic status.		no or limited prenatal care.	specimens at the day study consent was obtained and the day of delivery.	Right, Palpebral fissure width Left, Right, Inner canthal distance, Outer canthal distance, Philtrum, Internipple distance, Clitoral width, Clitoral length, Stretched penile length	possibly confounding variables		exposed group. Cocaine-exposed infants were significantly smaller in birth weight, length, and head circumference but did not differ on remaining anthropometric measurements.	interviewed after birth. 80% indicated that they used crack cocaine.
Aghamohammadi et al	2016 / marc 2011 – jan 2014	cohorte	Imam Khomeini hospital in Sari, Iran	women enrolled for delivery in the labor ward of Imam Khomeini hospital in Sari	Maternal sef-report and urine exams at the time of delivery	preeclampsia, placenta abruption, preterm labor, LBW and low Apgar score in 5 min		9	Preeclampsia 50% vs 28%; Placenta abruption 35% vs 14%; Preterm labor 61% vs 18%; Low birth weight 55% vs 25%; Low Apgar in 5 min 12% vs 14%	

According to the quality criteria, the NOS scores of all studies were all more than seven stars, indicating high quality (Table 3). Moreover, the ten articles included were classified as having a 'low risk of bias'. In the selection domain, two studies received three stars because the exposure assessment was not by secure record. In the comparability domain, two studies received one star for not describing a second important factor. In the exposure domain, one study received two stars because non-response rate was not clearly described (Table 3).

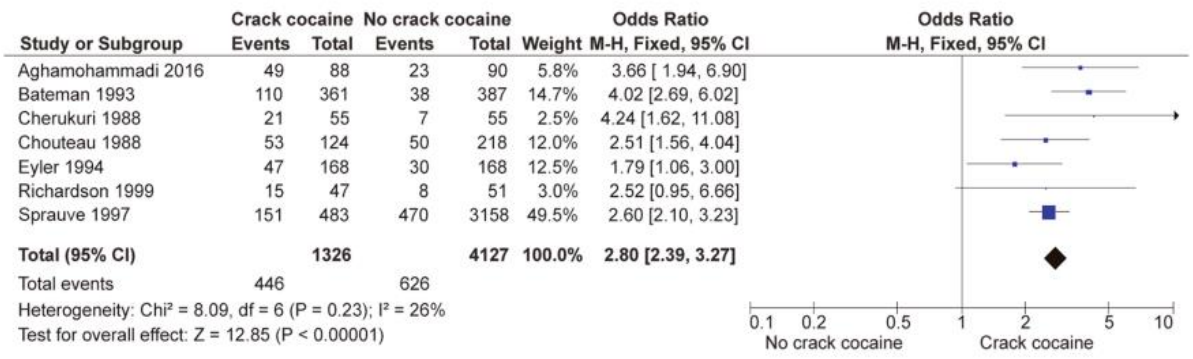
Table 3. Newcastle–Ottawa quality assessment scale

Case–control studies					
Author	Selection	Comparability	Exposure	Total stars	Risk of bias
Petitti et al. 1990	4	2	2	8	Low
Cohort studies					
Author	Selection	Comparability	Outcome	Total stars	Risk of bias
Bateman et al. 1993	4	2	3	9	Low
Eyler et al. 1998	4	2	3	9	Low
Richardson et al. 1999	3	2	3	8	Low
Cherukuri et al. 1988	3	1	3	7	Low
Eyler et al. 1994	4	2	3	9	Low
Aghamohammadi et al. 2016	4	2	3	9	Low
Sprauve et al. 1997	4	2	3	9	Low
Behnke et al. 2001	4	2	3	9	Low
Chouteau et al. 1988	4	1	3	8	Low

We performed a meta-analysis of the observed outcomes.

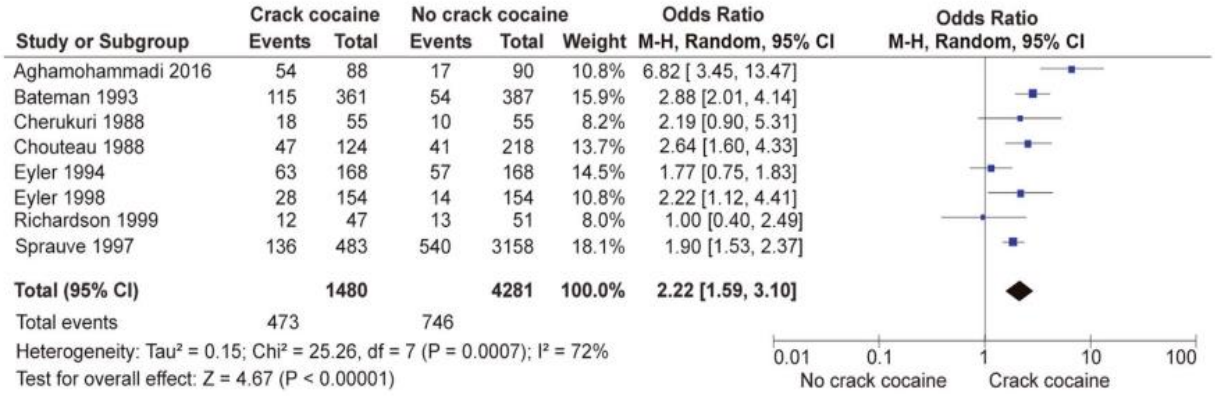
Low birth weight (LBW): crack cocaine use during pregnancy was significantly associated with LBW as compared with women who did not use crack cocaine during pregnancy (seven studies, OR, 2.80; 95% CI 2.39–3.27; I 2 26%) (Fig. 2).

Fig. 2 Effect of use of crack cocaine during the gestational period on low birth weight (<2500 g)



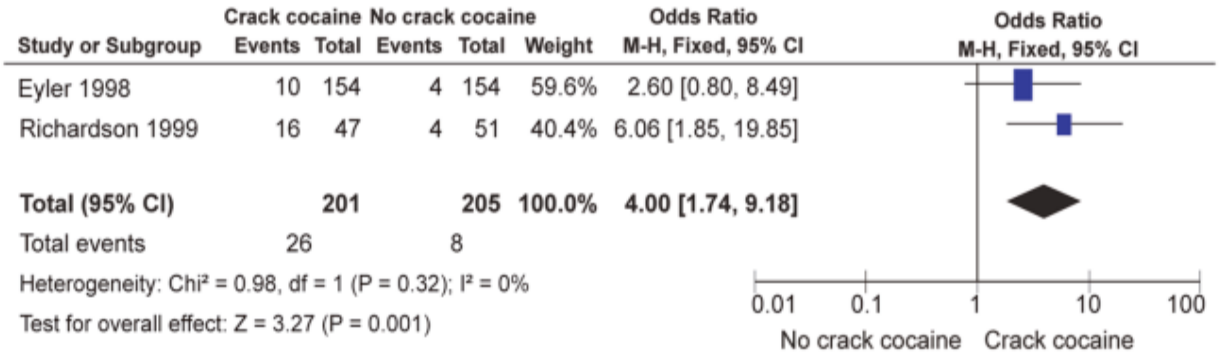
Preterm delivery: when compared with non-users, crack cocaine use during pregnancy was significantly associated with preterm delivery before 37 gestational weeks (eight studies, OR, 2.22; 95% CI 1.59– 3.10; I 2 72%) (Fig. 3).

Fig. 3 Effect of use of crack cocaine during the gestational period on preterm delivery (<37 weeks)



Small for gestational age (SGA): crack cocaine use during pregnancy vs no use was significantly associated with SGA (two studies, OR, 4.00; 95% CI 1.74–9.18; I 2 0%) (Fig. 4).

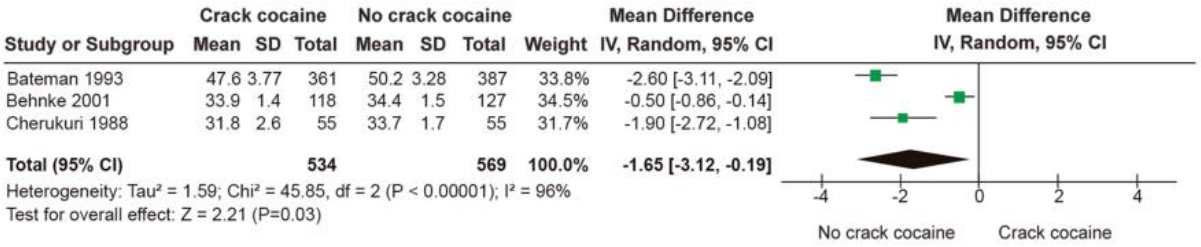
Fig. 4 Effect of use of crack cocaine during the gestational period on small for gestational age



Head circumference: crack cocaine use during pregnancy vs no use was significantly associated with smaller head circumference (three studies, −1.65 cm; 95% CI −3.12 to −0.19; I

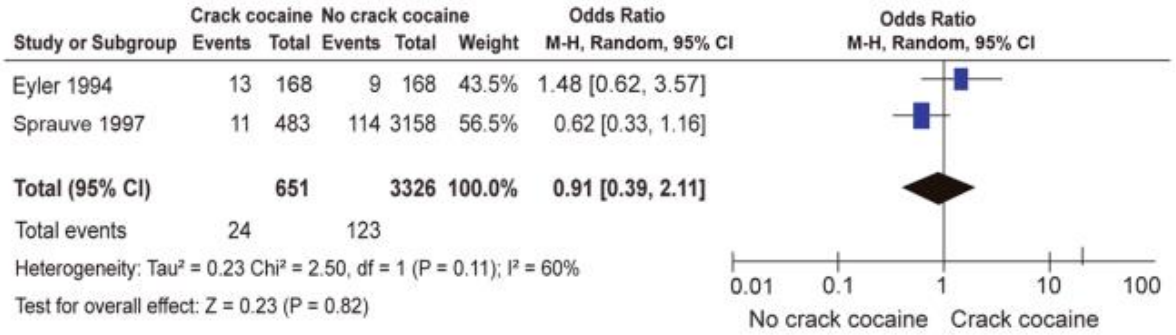
2 96%) (Fig. 5).

Fig 5 Effect of use of crack cocaine during the gestational period on head circumference



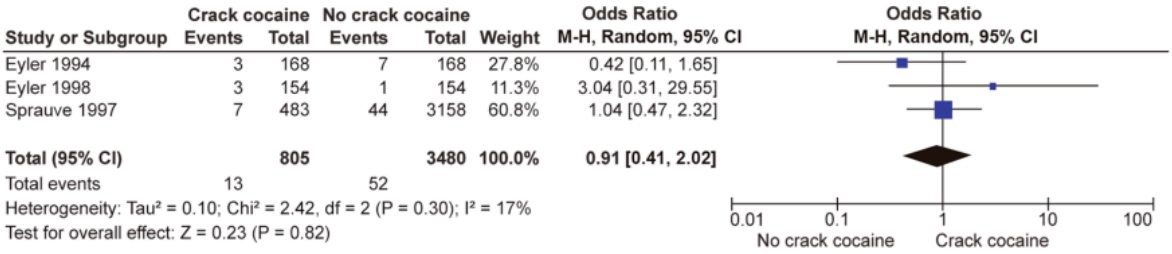
Congenital malformation: crack cocaine use during pregnancy vs no use was not associated with congenital malformation (two studies, OR, 0.91; 95% CI 0.39–2.11; I 2 60%) (Fig. 6).

Fig 6 Effect of use of crack cocaine during the gestational period on congenital malformation



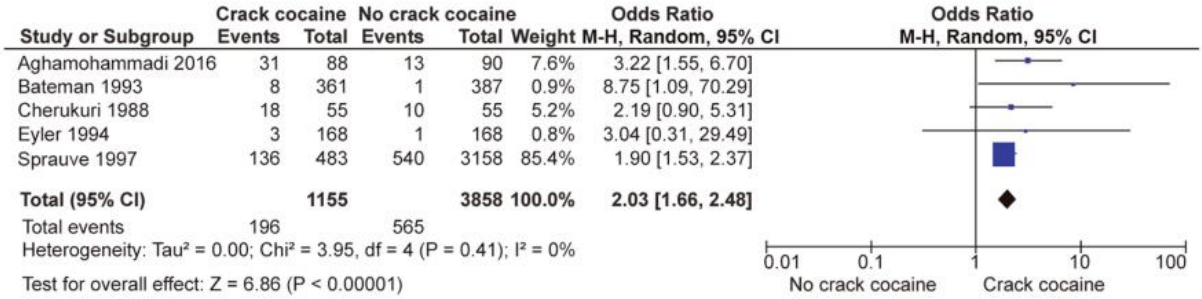
Fetal death: crack cocaine use during pregnancy vs no use was not associated with fetal death (three studies, 0.91; 95% CI 0.41–2.02; I 2 17%) (Fig. 7).

Fig 7 Effect of use of crack cocaine during the gestational period on fetal death



Placental displacement: crack cocaine use during pregnancy vs no use was signifcantly associated with placental displacement (five studies, 2.03; 95% CI 1.66–2.48; I 2 0%) (Fig. 8).

Fig 8 Effect of use of crack cocaine during the gestational period on placental displacement



Priori subgroup analyses were performed. Similar results were found for preterm delivery in both self-reported (OR = 3.53; 95% CI = 1.92–6.48; P < .005; I 2 = 65%) and objective-reported (OR = 1.76; 95% CI = 1.30–2.38; P < .005; I 2 = 50%); for placental displacement in both self-reported (OR = 2.97; 95% CI = 1.72–5.12; P < .005; I 2 =0%) and objective-reported (OR = 1.91; 95% CI = 1.53–2.37; P < .005; I 2 = 0%); for low birth weight in both self-reported (OR =3.95; 95% CI = 2.87–5.44; P < .005; I2 =0%) and objective-reported (OR = 2.47; 95% CI = 2.28–3.48; P < .005; I2 = 26%). This analysis was not done for the other variables since they were all of the same types. This analysis was not done prospectively or retrospectively because all the studies had a disposition for a retrospective study. We conducted sensitivity analyses model to evaluate the stability of the crude results by removing one study at each round of the analysis. We have observed that studies were not a greater source of heterogeneity.

4 DISCUSSION

To the best of our knowledge, this systematic review is a pioneer in evaluating gestational consequences of the crack cocaine consumption. Other reports that addressed this topic provide updates regarding other drugs or focused on cocaine in the free form [21 –24]. However, the original observational studies are not restricted to isolated crack cocaine, since the users often associate crack cocaine abuse with other drugs. Additionally, this type of research presents other variables such as experimental design and location and form of expo - sure. For this reason, the inclusion and exclusion factors in our review were very selective (Fig. 1), leaving few selected studies to be included in the current work. Nevertheless, we concluded that despite the small number of studies included in our analysis, there is sufficient evidence for establishing a clear association between crack cocaine gestational exposure and maternal, fetal and neonatal consequences.

The ten studies included in this review revealed that crack cocaine use during pregnancy had association with low birth weight [<2500 g (Fig. 2)], preterm delivery [before 37 weeks (Fig. 3)], small for gestational age (Fig. 4), head circumference (Fig. 5) and placental displacement (Fig. 8). On the other hand, our meta-analysis results do not suggest an association of exposition to crack cocaine during fetal development with a congenital malformation (Fig. 6) and fetal death (Fig. 7). Our analysis indicated that children of crack cocaine user mothers had a significant low birth weight and small for gestational age when compared with the drug-free groups. This is possibly associated with lack of nutrition due the gradual reduction in placental blood flow produced by vasoconstriction, leading to decrease in oxygen and nutrient transfer to the fetus [11, 14] and monoamine neurotransmitter functions of the developing fetal brain [25, 26]. Additionally, pregnant crack cocaine users usually present lack of appetite and are less engaged, which could contribute to maternal nutrition impairments [27 –34]. In fact, low birth weight is often evaluated on short- and long-term outcomes. In general, the short-term alterations are related to higher rates of mortality at birth, in addition to high medical costs [35]. However, Table our results do not indicate effects of maternal exposure of crack cocaine on mortality, maternal and newborn length of hospital stay or hospitalization in the neonatal intensive care unit. On the other hand, there is no clear correlation between long-term effects and low birth weight [36], which showed evidence that individuals exposed to crack cocaine in the gestational period are prone to heart disease and type 2 diabetes later in life. Additionally, other studies showed that low birth weight could impact on IQ, labor force participation, adverse economic outcomes and reduced mental performance [37–41]. However, when low

birth weight is due to prenatal crack cocaine exposure, adolescents present problems of self-regulation and poor impulse control [32, 42–45]. At the age of 14, they present two-fold more chances to use cocaine [46], and at 15, they have 1.8 times more tendency to alcohol and marijuana or tobacco use [32, 45, 47]. Despite these reports, new studies investigating these percentages in adulthood and the mechanism of action of placental flow culminating in low birth weight are needed, as well long-term studies to identify cognitive performance deficits in adulthood. Preterm birth represents a worldwide public health problem, especially in developing countries [48, 49]. Although preterm infants are more sensitive than term, because of complications from cerebral [50] and pulmonary [51] immaturity, mortality rates have been decreasing due to the use of new medical advances [52, 53]. Additionally, a preclinical study suggested that cocaine increases the viability of oxytocin (Oxt) in the pregnant baboon [54]. All these data corroborate with our results, indicating a higher incidence of preterm delivery in crack cocaine users, which can be attributed to the effect of crack cocaine increasing catecholamine levels [14], resulting in augmented sensibility to Oxt via adrenergic receptors, leading to uterine constriction. However, the mechanism by which cocaine induces alterations in Oxt levels is not fully understood [55]. Thereby, the variation in Oxt levels may explain the high rates of preterm births observed in our analysis. Other studies are needed with the Oxt antagonist, for example, to verify if it would be useful in relaxing myometrial contractions, which in turn, could avoid the preterm delivery in pregnant crack cocaine users. Other recent study indicated that women who suspended the use of crack cocaine in the first trimester of pregnancy presented high rates of preterm delivery [56], indicating that probably, the increased of Oxt is not dependent of chronic use. Gestational intoxication to drugs of abuse leads to several behavioral and neurobiological alterations, radically increasing children's vulnerability to cognition defcits [57–60]; however, these alterations in prenatal cocaine exposure in rodents and humans are dose, sex-, and agedependent [61–63]. Interestingly, impairments in cognitive performance and behavior are modulated by Oxt, signaling that the stress response is preventing social interactions, and thereby increasing the risk of drug abuse [55]. In addition to Oxt, other factors have also been associated with preterm birth, including infection [64], uterine overdistension [48], low socioeconomic status [65] and smoking [66]. However, new clinical and preclinical studies should be developed to investigate the role of Oxt in the observed high incidence of preterm delivery in prenatal crack cocaine exposure.

Our results indicate that perinatal exposure to crack cocaine, as well as intranasal cocaine exposure, has an impact on brain development, typified by reduced head circumference (HC) [24, 67]. Although inferences based on HC or occipitofrontal circumference must be

conducted with caution, it is a valuable tool used as a measure of neurological development in newborn, infancy and childhood [68, 69], besides being a fast and inexpensive way to identify abnormalities on cognitive functions [70–72], intracranial volume [73], sex- and age-dependent brain volume [74] and more than 500 conditions associated with microcephaly and macrocephaly [75, 76]. Animal studies with prenatal administration of cocaine have been shown alterations in neocortex [25, 77–80], hippocampus [81], striatum, thalamus, globus pallidus [82] and disruptions in myelination [83]. Although these studies did not directly evaluate the HC, such alterations may be related to a reduced HC in humans [84]. Unfortunately, there are no animal models of perinatal crack cocaine exposure to understand if other routes of administration of cocaine (crack cocaine) generate similar effects. Probably, the alterations indicated in our results about reduced HC by exposure to crack would be even more devastating due the active compound, anhydroecgonine methyl ester (AEME), generated from cocaine pyrolysis, which has been associated with a higher risk of neurotoxicity and greater addiction [1, 85]. All these data emphasize the need for new clinical and experimental studies to elucidate the influence of the smoked form of cocaine on the aforementioned alterations.

Another significant finding from our results is the association of crack cocaine use and placental abruption. This pathology is associated with late pregnancy bleeding, uterine contraction and pain [86, 87]. The mortality ranges from 20 to 67% depending on fetal weight, gestational age and degree of abruption [88]. Several studies indicate the risk factors for placental abruption including maternal age, multiple gestation, chronic hypertension, premature membrane rupture, oligohydramnios dietary or nutritional deficiency, cigarette smoking, cocaine and crack cocaine drug use [11, 89–101]. The animal study developed by Wu and colleagues [102] showed that prenatal cocaine exposure could disrupt pathways of the regulation for placental progesterone synthesis associated with the declining level of progesterone in both maternal and fetal plasma. The maintenance of high levels of progesterone in the luteal phase (human cycle) is necessary because it stimulates estradiol (E2)-primed human endometrial stromal cells to decidualized and express tissue factor (TF) [103] that prevents hemorrhage [104] associated with severe placental abruption [105]. Although crack cocaine is one clear risk factor, there are inconclusive studies developed in human and no other studies in animal models. In this way, new studies are needed to establish a clear relationship between crack cocaine prenatal exposure and placental abruption.

Our data did not suggest an association between intrauterine growth restriction and preeclampsia followed by prenatal crack cocaine exposure. In fact, it was not possible to perform a meta-analysis, because only two articles included in this review addressed these

variables. However, they reported increased risk for intrauterine growth restriction (29% users vis-a-vis 13% non-users) [99] and influence of crack cocaine, during pregnancy, on the prevalence of preeclampsia (50% users vs 28% non-users) [11]. Additionally, our results suggested in most cases alteration related to newborn and fetus; however, new studies are needed to confirm and understand the mechanisms by which crack cocaine/ cocaine would lead to preeclampsia, since this alteration can cause preterm lung maturation [106], consequently increasing the risk of neonatal death. The similarity to intrauterine restriction and preeclampsia, fetal death and congenital malformation was not statistically associated with crack cocaine use during pregnancy. Behnke et al. (2001) [107] evaluated congenital malformation after prenatal cocaine exposure, and although intoxicated infants presented lower birth weight and smaller head circumference compared with drug-free infants, they did not identify other anthropometric alterations in the number of significant or minor anomalies that are characteristic of congenital malformation.

Collectively, our results reinforce the known harmful effects of cocaine on mother and fetus. Also, the use of the smoked form of cocaine can be considered even more dangerous because of the main product of the pyrolysis of cocaine AEME [1, 108, 109]. AEME is exclusively produced when cocaine pyrolysis occurs, and it is an analytical marker for crack cocaine consumption [110–113]. This compound potentiates the neurotoxic effects of cocaine, leading to impairments on long-term spatial working memory and increased oxidation and antioxidant enzymes levels in the striatum [114]. In another in vivo study, Garcia et al. (2017) [108] reported that AEME increased the locomotor activity and dopamine in reward circuitry, emphasizing the addiction power of this substance. Despite the increasing number of studies on AEME, the effects of this substance during pregnancy are still completely unknown. In this direction, clinical studies could establish the consequences of AEME exposition due to the use of crack cocaine during the gestational period. Additionally, new preclinical studies with prenatal exposure to crack cocaine smoke or isolated AEME compound are crucial for a better understanding of the prenatal consequences of crack cocaine use.

Limitations

This review intended to verify the effects of crack cocaine on pregnancy. To overcome typical limitations of this kind of work, we chose to use an open (using general terms) search strategy, leaving the restrictive phase to the selections based on titles and abstract and complete article readings. An assured result was that all ten selected articles included in this review showed a low risk of bias, according to the NOS quality assessment. On the other hand, an important restriction that must be considered when interpreting the results of all studies on human drug abuse is that crack cocaine addicted people usually have contact with other abuse drugs; the results presented may be contaminated by other drugs effects. Additionally, the crack cocaine use was recognized exclusively by questionnaire, leaving a relative uncertainty on the dose to which the mothers were exposed, as well as the frequency of drug use, adding variability to the severity of the effects observed. This emphasizes the need for more new and controlled studies, both in human subjects and in animal models, to better understand the specific effects of crack cocaine on pregnancy and fetal and infant outcomes.

5 CONCLUSION

Crack cocaine use has become a severe public health issue in the world [115–119]. While one study indicates stabilization on the number of crack cocaine users in the USA [120], another study suggests a trend towards the use of crack cocaine among other drug users in Canada [121]. In Brazil, increasing rates of crack cocaine-related hospitalizations (from 2.8% in 1997 to 67.8% in 2010) among women, due to psychoactive drugs use, was reported [122]. Recent articles have shown that the effects of crack on mothers and their in utero crack-exposed children are very different [123, 124] and that this exposition can continue after birth, both via breastfeeding and passive inhalation, resulting in long-term effects of the drug on the child's organism [125]. For instance, newborns exposed to crack cocaine in utero express neuroprotective and increased levels of neurotrophic factors, while mothers do not [123, 124]. Despite the expression of such possible protective factors, children with prenatal cocaine exposure are at a greater risk for developing learning and language disabilities [126, 127], as well as increased caregiver-reported behavioral problems [128]. Moreover, crack cocaine-addicted women show increased impulsivity [129] and low performances on executive function [130], behavioral modifications that may be linked to the commonly observed sex exchange for

crack cocaine among female users [131–133], what can be directly related to the here reviewed complicated pregnancies and children health issues related to crack cocaine use. This work provides sound evidence for the deleterious effects of crack cocaine use on the mother's and fetus' health, confirmed by meta-analysis. Such adverse effects are evidenced in exposed fetuses by smaller size for the gestational age, in utero exposed newborns by lower birth weights and head circumference, and in crack cocaine using pregnant women by higher occurrence of placental displacement and preterm delivery. Increasing endeavors must be made on comprehending the outcomes of crack cocaine use, both to mothers and children, to address the health and social consequences of this behavior.

Author contributions JFS: data collection, project development, manuscript writing and editing, data administration, data interpretation. CMBC: data collection, project development, manuscript writing and editing, data administration, data interpretation. MD: manuscript editing. DLGG: manuscript editing. CQT: manuscript editing. FTB: project development, data administration, data interpretation, data analysis, statistical analysis. AKS: manuscript editing. OWC: project development, scientific knowledge, data collection, project development manuscript writing and editing

Compliance with ethical standards

Conflict of interest

All authors declare that they have no conflict of interest.

References

1. Garcia RCT, Dati LMM, Fukuda S et al (2012) Neurotoxicity of anhydroecgonine methyl ester, a crack cocaine pyrolysis product. Toxicol Sci 128:223–234

2. Hatsukami DK, Fischman MW (1996) Crack cocaine and cocaine hydrochloride. Are the differences myth or reality? JAMA 276:1580–1588

3. Ribeiro M, Dunn J, Sesso R et al (2006) Causes of death among crack cocaine users. Rev Bras Psiquiatr 28:196–202

4. Narvaez JCM, Jansen K, Pinheiro RT et al (2014) Violent and sexual behaviors and lifetime use of crack cocaine: a population-based study in Brazil. Soc Psychiatry Psychiatr Epidemiol 49:1249–1255

5. Friedman AS, Glassman K, Terras BA (2001) Violent behavior as related to use of marijuana and other drugs. J Addict Dis 20:49–72

6. Riezzo I, Fiore C, De Carlo D et al (2012) Side effects of cocaine abuse: multiorgan toxicity and pathological consequences. Curr Med Chem 19:5624–5646

7. Duailibi LB, Ribeiro M, Laranjeira R (2008) Profile of cocaine and crack users in Brazil. Cad Saude Publ 24:s545–s557

8. Bell GL, Lau K (1995) Perinatal and neonatal issues of substance abuse. Pediatr Clin North Am 42:261–281

9. Ganapathy V (2011) Drugs of abuse and human placenta. Life Sci 88:926–930

10. Richardson GA, Goldschmidt L, Larkby C (2007) Effects of prenatal cocaine exposure on growth: a longitudinal analysis. Pediatrics 120:e1017–e1027

11. Aghamohammadi A, Zafari M (2016) Crack abuse during pregnancy: maternal, fetal and neonatal complications. J Matern Fetal Neonatal Med 29:795–797

12. Burkett G, Bandstra ES, Cohen J et al (1990) Cocaine-related maternal death. Am J Obstet Gynecol 163:40–41

13. Dow-Edwards D (2010) Sex diferences in the efects of cocaine abuse across the life span. Physiol Behav 100(3):208–215

14. Legido A, Clancy RR, Spitzer AR, Finnegan LP (1992) Electroencephalographic and behavioral-state studies in infants of cocaine-addicted mothers. Am J Dis Child 146:748–752

15. Kessler F, Pechansky F (2008) A psychiatric view on the crack phenomenon nowadays. RevKesslerista Psiquiatr do Rio Gd do Sul 30:96–98

16. Volpe JJ (1992) Effect of cocaine use on the fetus. N Engl J Med 327:399–407

17. Moher D, Liberati A, Tetzlaf J et al (2010) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg 8:336–341

18. Stroup DF, Berlin JA, Morton SC et al (2000) Meta-analysis of observational studies. J Am Med Assoc 283:2008–2012

19. Wells G, Shea B, O'Connell D, et al The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 27 Jun 2017

20. Wu QJ, Yang Y, Vogtmann E et al (2013) Cruciferous vegetables intake and the risk of colorectal cancer: a meta-analysis of observational studies. Ann Oncol 24:1079–1087

21. Addis A, Moretti ME, Ahmed Syed F et al (2001) Fetal efects of cocaine: an updated meta-analysis. Reprod Toxicol 15:341–369

22. De Castro AP, Cristina M, Guilam R et al (2013) Violência na velhice: abordagens em periódicos nacionais indexados Violence in old age: the issue addressed in indexed national journals. Ciência e Saúde Coletiva 18:1283–1292

23. Frank DA, Augustyn M, Knight WG, Pell T, Zuckerman B (2001) Growth, development, and behavior in early childhood following prenatal cocaine exposure: a systematic review. JAMA 285:1613–1625

24. Gouin K, Murphy K, Shah PS (2011) Efects of cocaine use during pregnancy on low birthweight and preterm birth: Systematic review and metaanalyses. Am J Obstet Gynecol 204:340.e1–340.e12

25. Kosofsky BE, Wilkins AS, Gressens P, Evrard P (1994) Transplacental cocaine exposure: a mouse model demonstrating neuroanatomic and behavioral abnormalities. J Child Neurol 9:234–241

26. McCarthy DM, Kabir ZD, Bhide PG, Kosofsky BE (2014) Efects of prenatal exposure to cocaine on brain structure and function. Prog Brain Res 211:277–289

27. Fajemirokun-Odudeyi O, Lindow SW (2004) Obstetric implications of cocaine use in pregnancy: a literature review. Eur J Obstet Gynecol Reprod Biol 112:2–8

28. Burns K, Chethik L, Burns WJ, Clark R (1991) Dyadic disturbances in cocaine abusing mothers and their infants. J Clin Psychol 47:316–319

29. Eiden RD, Stevens A, Schuetze P, Dombkowski LE (2006) Conceptual model for maternal behavior among polydrug cocaine using mothers: the role of postnatal cocaine use and maternal depression. Psychol Addict Behav 20:1–10

30. Tronick EZ, Messinger DS, Weinberg MK et al (2005) Cocaine exposure is associated with subtle compromises of infants' and mothers' social-emotional behavior and dyadic features of their interaction in the face-to-face still-face paradigm. Dev Psychol 41:711–722

31. Black M, Nair P, Kight C et al (1994) Parenting and early development among children of drug abusing women: efects of home intervention. Pediatrics 94(4 Pt 1):440–448

32. Minnes S, Singer L, Min MO et al (2014) Efects of prenatal cocaine/polydrug exposure on substance use by age 15. Drug Alcohol Depend 134:201–210

33. Minnes S, Singer LT, Arendt R, Satayathum S (2005) Efects of prenatal cocaine/polydrug use on maternal-infant feeding interactions during the first year of life. Nat Rev Mol Cell Biol 26:194–200

34. Garland OH (1895) Fatal acute poisoning by cocaine. Lancet 146:1104–1105

35. Almond D, Chay K, Lee D (2005) The Costs of Low Birth Weight. Q J Econ 120:1031–1083

36. Barker DJ (1995) Fetal origins of coronary heart disease. BMJ Br Med J 311:171

37. Black SE, Devereux JP, Salvanes KG (2007) From the cradle to the labor market? the effect of birth weight on adult outcomes. Q J Econ 122(1):409–439

38. Schulz LC (2010) The Dutch hunger winter and the developmental origins of health and disease. Proc Natl Acad Sci 107:16757–16758

39. Mazumder B, Almond D (2011) Health capital and the prenatal environment: the effect of maternal fasting during pregnancy. FRB of Chicago Working Paper No. 2007–22 1–74

40. de Rooij SR, Wouters H, Yonker JE et al (2010) Prenatal undernutrition and cognitive function in late adulthood. Proc Natl Acad Sci 107:16881–16886

41. Stein Z, Susser M, Saenger G, Marolla F (1975) Famine and human development: the Dutch hunger winter of 1944–1945. Oxford University Press, New York, NY, US, pp 1–284

42. Singer LT, Nelson S, Short E et al (2008) Prenatal cocaine exposure: drug and environmental efects at 9 years. J Pediatr 153:105–111

43. Thompson BL, Levitt P, Stanwood GD (2009) Prenatal exposure to drugs: efects on brain development and implications for policy and education. 2 Nat Rev Neurosci 10:303–312

44. Minnes S, Singer LT, Kirchner HL et al (2010) The efects of prenatal cocaine exposure on problem behavior in children 4-10 years. Neurotoxicol Teratol 32:443–451

45. Minnes S, Min M, Wu M et al (2014) Executive function in adolescents with prenatal exposure to cocaine. Neurotoxicol Teratol 43:84

46. Greenwald MK, Chiodo LM, Hannigan JH et al (2011) Teens with heavy prenatal cocaine exposure respond to experimental social provocation with escape not aggression. Neurotoxicol Teratol 33:198–204

47. Richardson GA, Larkby C, Goldschmidt L, Day NL (2013) Adolescent initiation of drug use: efects of prenatal cocaine exposure. J Am Acad Child Adolesc Psychiatry 52:37–46

48. Goldenberg RL, Culhane JF, Iams JD, Romero R (2009) Preterm birth 1: epidemiology and causes of preterm birth. Obstet Anesth Dig 29:6–7

49. Purisch SE, Gyamf-Bannerman C (2017) Epidemiology of preterm birth. Semin Perinatol 41(7):387–391

50. Rees S, Inder T (2005) Fetal and neonatal origins of altered brain development. Early Hum

Dev 81:753–761

51. Jobe AH, Bancalari E (2001) NICHD/NHLBI/ORD workshop summary. Am J Respir Crit Care Med 163:1723–1729

52. Saigal S, Doyle LW (2008) [Commentary on] The lancet series on preterm birth (3): an overview of mortality and sequelae of preterm birth from infancy to adulthood. Child Care Heal Dev 34:407–408

53. Doyle LW, Saigal S (2009) Long-term outcomes of very preterm or tiny infants. Neoreviews 10:e130–e137

54. Morgan MA, Honnebier MBOM, Mecenas C, Nathanielsz PW (1996) Cocaine's effect on plasma oxytocin concentrations in the baboon during late pregnancy. Am J Obstet Gynecol 174:1026–1027

55. Williams SK, Johns JM (2014) Prenatal and gestational cocaine exposure: efects on the oxytocin system and social behavior with implications for addiction. Pharmacol Biochem Behav 119:10–21

56. Petitti DB, Coleman C (1990) Cocaine and the risk of low birth weight. Am Public Heal Assoc 80:25–28

57. Glatt SJ, Bolanos CA, Trksak GH et al (2000) Prenatal cocaine exposure alters behavioral and neurochemical sensitization to amphetamine in adult rats. Neuropharmacology 39:599–610

58. Chae S-M, Covington CY (2009) Biobehavioral outcomes in adolescents and young adults prenatally exposed to cocaine: evidence from animal models. Biol Res Nurs 10:318–330

59. Buckingham-Howes S, Berger SS, Scaletti LA, Black MM (2013) Systematic review of prenatal cocaine exposure and adolescent development. Pediatrics 131:e1917–e1936

60. Behnke M, Smith VC (2013) Prenatal substance abuse: short- and long-term efects on the exposed fetus. Pediatrics 131:e1009–e1024

61. Overstreet DH, Moy SS, Lubin DA et al (2000) Enduring efects of prenatal cocaine administration on emotional behavior in rats. Physiol Behav 70:149–156

62. Liu J, Lester BM (2011) Reconceptualizing in a dual-system model the efects of prenatal cocaine exposure on adolescent development: a short review. Int J Dev Neurosci 29:803–809
63. Lambert BL, Bauer CR (2012) Developmental and behavioral consequences of prenatal cocaine exposure: a review. J Perinatol 32:819–828

64. Henderson JJ, McWilliam OA, Newnham JP, Pennell CE (2012) Preterm birth a etiology 2004-2008. Maternal factors associated with three phenotypes: spontaneous preterm labour, preterm prelabour rupture of membranes and medically indicated preterm birth. J Matern Fetal Neonatal Med 25:642–647

65. Smith LK, Draper ES, Manktelow BN et al (2007) Socioeconomic inequalities in very preterm birth rates. Arch Dis Child Fetal Neonatal Ed 92:F11–F14

66. Ion R, Bernal AL (2015) Smoking and preterm birth. Reprod Sci 22:918–926

67. Nordstrom-Klee B, Delaney-Black V, Covington C et al (2002) Growth from birth onwards of children prenatally exposed to drugs: a literature review. Neurotoxicol Teratol 24:481–488

68. Treit S, Zhou D, Chudley AE et al (2016) Relationships between head circumference, brain volume and cognition in children with prenatal alcohol exposure. PLoS One 11:1–15

69. Rollins JD, Collins JS, Holden KR (2010) United States head circumference growth reference charts: birth to 21 years. J Pediatr 156(6):907–913.e2

70. Gale CR, Walton S, Martyn CN (2003) Foetal and postnatal head growth and risk of cognitive decline in old age. Brain Epub 126(Pt 10):2273–2278

71. Gale CR, O'Callaghan FJ, Godfrey KM et al (2004) Critical periods of brain growth and cognitive function in children. Brain 127:321–329

72. Gale CR, O'Callaghan FJ, Bredow M, Martyn CN (2006) The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. Pediatrics 118:1486–1492

73. Bray PF, Shields WD, Wolcott GJ, Madsen JA (1968) Occipitofrontal head circumference—an accurate measure of intracranial volume. J Pediatr 75:3–5

74. Bartholomeusz HH, Courchesne E, Karns CM (2002) Relationship between head circumference and brain volume in healthy normal toddlers, children, and adults. Neuropediatrics 33:232–238

75. Holden K, Lyons M (2009) Acquired microcephaly. In: Maria BL E (ed) Current management in child neurology, 4th ed. Canada

76. Williams CA, Dagli A, Battaglia A (2008) Genetic disorders associated with macrocephaly. Am J Med Genet Part A 146:2023–2037

77. He N, Lidow MS (2004) Cerebral cortical abnormalities seen in a non-human primate model of prenatal cocaine exposure are not related to vasoconstriction. Neurotoxicology 25:419–432

78. Jones L, Fischer I, Levitt P (1996) Nonuniform alteration of dendritic development in the cerebral cortex following prenatal cocaine exposure. Cereb Cortex 6:431–445

79. Lidow MS, Song ZM (2001) Primates exposed to cocaine in utero display reduced density and number of cerebral cortical neurons. J Comp Neurol 435:263–275

80. Lidow MS, Song ZM (2001) Effect of cocaine on cell proliferation in the cerebral wall of monkey fetuses. Cereb Cortex 11:545–551

81. Baraban SC, Wenzel HJ, Castro PA, Schwartzkroin PA (1999) Hippocampal dysplasia in rats exposed to cocaine in utero. Dev Brain Res 117:213–217

82. Mcmurray MS, Oguz I, Rumple AM et al (2015) Efects of prenatal cocaine exposure on early postnatal rodent brain structure and diffusion properties. Neurotoxicol Teratol 47:80–88
83. Wiggins RC, Ruiz B (1990) Development under the influence of cocaine. II. Comparison of the efects of maternal cocaine and associated undernutrition on brain myelin development in the offspring. Metab Brain Dis 5:101–109

84. Bateman DA, Chiriboga CA (2000) Dose-response effect of cocaine on newborn head circumference. Pediatrics 106:E33

85. Areal LB, Rodrigues LCM, Andrich F et al (2015) Behavioural, biochemical and molecular changes induced by chronic crack cocaine inhalation in mice: the role of dopaminergic and endocannabinoid systems in the prefrontal cortex. Behav Brain Res 290:8–16

86. Sakornbut E, Leeman L, Fontaine P (2007) 5—Late pregnancy bleeding. Am Fam Physician 75:1199–1206

87. Fiori O, Verstraete L, Berkane N (2007) Risk factors of abruptio placentae among Peruvian women. Am J Obstet Gynecol 196:e15

88. Ananth CV, Getahun D, Peltier MR, Smulian JC (2006) Placental abruption in term and preterm gestations: evidence for heterogeneity in clinical pathways. Obstet Gynecol 107:785–792

89. Ananth CV, Smulian JC, Demissie K et al (2001) Placental abruption among singleton and twin births in the United States: risk factor profiles. Am J Epidemiol 153:771–778

90. Ananth CV, Savitz DA, Williams MA (1996) Placental abruption and its association with hypertension and prolonged rupture of membranes: a methodologic review and meta-analysis. Obstet Gynecol 88:309–318

91. Ananth CV, Savitz DA, Luther ER (1996) Maternal cigarette smoking as a risk factor for placental abruption, placenta previa, and uterine bleeding in pregnancy. Am J Epidemiol 144:881–889

92. Ananth CV, Savitz DA, Bowes WA, Luther ER (1997) Influence of hypertensive disorders and cigarette smoking on placental abruption and uterine bleeding during pregnancy. Br J Obstet Gynaecol 104:572–578

93. Ananth CV, Oyelese Y, Srinivas N et al (2004) Preterm premature rupture of membranes, intrauterine infection, and oligohydramnios: risk factors for placental abruption. Obstet Gynecol 104:71–77

94. Sheiner E, Shoham-Vardi I, Hallak M et al (2003) Placental abruption in term pregnancies: clinical significance and obstetric risk factors. J Matern Fetal Neonatal Med 13:45–49

95. Salihu HM, Bekan B, Aliyu MH et al (2005) Perinatal mortality associated with abruptio placenta in singletons and multiples. Am J Obstet Gynecol 193:198–203

96. Bateman DA, Ng SK, Hansen CA, Hegarty MC (1993) The efects of intrauterine cocaine exposure in newborns. Rev Mal Respir 83:190–193

97. Cherukuri R, Minkof H, Feldman J et al (1988) A cohort study of alkaloidal cocaine (“crack”) in pregnancy. Obstet Gynecol 72:147–151

98. Eyler FD, Behnke M, Conlon M et al (1994) Prenatal cocaine use: a comparison of neonates matched on maternal risk factors. Neurotoxicol Teratol 16:81–87

99. Sprauve ME, Lindsay MK, Herbert S, Graves W (1997) Adverse perinatal outcome in

parturients who use crack cocaine. Obstet Gynecol 89:674–678

100. Aliyu MH, Lynch O, Wilson RE et al (2011) Association between tobacco use in pregnancy and placenta-associated syndromes: a population-based study. Arch Gynecol Obstet 283:729–734

101. Agarwal R, Gupta B, Radhakrishnan G (2011) Rupture of intrapartum unscarred uterus at the fundus: a complication of passive cocaine abuse? Arch Gynecol Obstet 283:53–54

102. Wu L, Yan J, Qu SC et al (2012) Abnormal regulation for progesterone production in placenta with prenatal cocaine exposure in rats. Placenta 33:977–981

103. Lockwood CJ, Krikun G, Papp C et al (1994) The role of progestationally regulated stromal cell tissue factor and type-1 plasminogen activator inhibitor (PAI-1) in endometrial hemostasis and menstruation. Ann N Y Acad Sci 734:57–79

104. Moore KL (1988) Formation of the bilaminar embryo. In: Wonsiewicz M (ed) The developing human: clinically oriented embryology, vol 4. W.B. Saunders, Philadelphia, pp 38–49

105. Pritchard JA, Wright MR (1959) Pathogenesis of hypofbrinogenemia in placental abruption. N Engl J Med 261:218–222

106. Chasnof IJ, Lewis DE, Grifth DR, Willey S (1989) Cocaine and pregnancy: clinical and toxicological implications for the neonate. Clin Chem 35:1276–1278

107. Behnke M, Eyler FD, Garvan CW, Wobie K (2001) The search for congenital malformations in newborns with fetal cocaine exposure. Pediatrics 107:E74

108. Garcia RCT, Torres LH, Balestrin NT et al (2017) Anhydroecgonine methyl ester, a cocaine pyrolysis product, may contribute to cocaine behavioral sensitization. Toxicology 376:44–50

109. Jacob P, Jones RT, Benowitz NL et al (1990) Cocaine smokers excrete a pyrolysis product, anhydroecgonine methyl ester. J Toxicol Clin Toxicol 28:121–125

110. Kintz P, Cirimele V, Sengler C, Mangin P (1995) Testing human hair and urine for anhydroecgonine methyl ester, a pyrolysis product of cocaine. J Anal Toxicol 19:479–482 111. Martin BR, Lue LP, Boni JP (1989) Pyrolysis and volatilization of cocaine. J Anal Toxicol 13:158–162

112. Toennes SW, Fandiño AS, Hesse F-J, Kauert GF (2003) Artifact production in the assay of anhydroecgonine methyl ester in serum using gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 792:345–351

113. Toennes SW, Fandiño AS, Kauert G (1999) Gas chromatographic-mass spectrometric detection of anhydroecgonine methyl ester (methylecgonidine) in human serum as evidence of recent smoking of crack. J Chromatogr B Biomed Sci Appl 735:127–132

114. Gomes EF, Lipaus IFS, Martins CW et al (2017) Anhydroecgonine methyl ester (AEME), a product of cocaine pyrolysis. Impairs Spatial Working Memory and Induces Striatal Oxidative Stress in Rats, Neurotox Res

115. UNODC (2016) United Nations ofce on drugs and crime. In: Report WD (ed) UN ofce on drugs and crime. World Drug Report, Vienna

116. Miguel AQC, Madruga CS, Cogo-Moreira H et al (2016) Contingency management is effective in promoting abstinence and retention in treatment among crack cocaine users in Brazil: a randomized controlled trial. Psychol Addict Behav 30:536–543

117. Des Jarlais DC, McKnight C, Arasteh K et al (2014) A perfect storm: crack cocaine, HSV-2, and HIV among non-injecting drug users in New York city. Subst Use Misuse 49:783–792

118. DeBeck K, Buxton J, Kerr T et al (2011) Public crack cocaine smoking and willingness to use a supervised inhalation facility: implications for street disorder. Subst Abuse Treat Prev Policy 6:4

119. Sterk CE, Elifson KW, DePadilla L (2014) Neighbourhood structural characteristics and crack cocaine use: exploring the impact of perceived neighborhood disorder on use among African Americans. Int J Drug Policy 25:616–623

120. Parker MA, Anthony JC (2014) Should anyone be riding to glory on the now-descending limb of the crack-cocaine epidemic curve in the United States? Drug Alcohol Depend 138:225–228

121. Werb D, Debeck K, Kerr T et al (2010) Modeling crack cocaine use trends over 10 years in a Canadian setting. Drug Alcohol Rev 29:271–277

122. Moreira MM, Barbosa GL, Laranjeira R, Mitsuhiro SS (2014) Alcohol and Crack cocaine use in women: a 14-year cross-sectional study. J Addict Dis 33:9–14

123. Parcianello RR, Mardini V, Ceresér KMM et al (2018) Increased cocaine and amphetamine-regulated transcript cord blood levels in the newborns exposed to crack cocaine in utero. Psychopharmacol 235(1):215–222

124. Mardini V, Rohde LA, Ceresér KM et al (2017) TBARS and BDNF levels in newborns exposed to crack/cocaine during pregnancy: a comparative study. Rev Bras Psiquiatr 39:263–266

125. Jones W (2015) Cocaine use and the breastfeeding mother. Pract Midwife 18:19–22

126. Morrow CE, Culbertson JL, Accornero VH et al (2006) Learning disabilities and intellectual functioning in school-aged children with prenatal cocaine exposure. Dev Neuropsychol 30:905–931

127. Bandstra ES, Vogel AL, Morrow CE et al (2004) Severity of prenatal cocaine exposure and child language functioning through age 7 years: a longitudinal latent growth curve analysis. Subst Use Misuse 39:25–59

128. Warner TD, Behnke M, Hou W et al (2006) Predicting caregiver reported behavior problems in cocaine-exposed children at 3 years. J Dev Behav Pediatr 27:83–92

129. Hess ARB, Menezes CB, de Almeida RMM (2018) Inhibitory control and impulsivity levels in women crack users. Subst Use Misuse 53(6):972–979

130. Levandowski ML, Hess ARB, Grassi-Oliveira R, de Almeida RMM (2016) Plasma interleukin-6 and executive function in crack cocaine-dependent women. Neurosci Lett 628:85–90

131. Duf P, Tyndall M, Buxton J et al (2013) Sex-for-crack exchanges: associations with risky sexual and drug use niches in an urban Canadian city. Harm Reduct J 10:29

132. Vernaglia TVC, Vieira RA de MS, Cruz MS (2015) Crack cocaine users living on the streets – gender characteristics. Cien Saude Colet 20:1851–1859

133. Kopetz CE, Collado A, Lejuez CW (2015) When the end (automatically) justifes the means: automatic tendency toward sex exchange for crack cocaine. Motiv Sci 1:233–244

134. Chouteau M, Namerow PB, Leppert P (1988) The effect of cocaine abuse on birth weight and gestational age. Obstet Gynecol 72(3 Pt 1):351–354

135. Eyler FD, Behnke M, Conlon M et al (1998) Birth outcome from a prospective, matched study of prenatal crack/cocaine use: I. Interactive and dose efects on health and growth. Pediatrics 101:229–237

136. Richardson GA, Hamel SC, Goldschmidt L, Day NL (1999) Growth of infants prenatally exposed to cocaine/crack: comparison of a prenatal care and a no prenatal care sample. Pediatrics 104:e18

RESUMO - ARTIGO 2

O consumo de drogas de abuso é um dos principais desafios de saúde pública levando a diversos efeitos adversos. Os efeitos fisiológicos das drogas de abuso são estudados em modelos animais e a *Drosophila melanogaster* se destaca nesse tipo de pesquisa. O objetivo principal deste trabalho foi avaliar as alterações causadas por drogas de abuso em *D. melanogaster*, uma vez que este tem sido um modelo bem estudado e que pode ajudar a compreender os mecanismos subjacentes da dependência de drogas em humanos. Foi realizada uma revisão sistemática com metanálise e a busca foi realizada em 7 bancos de dados online (PubMed, EMBASE, ISI Web of Science, LILACS, SIGLE, Science Direct e Google Scholar), desde o início de cada banco de dados até janeiro de 2023, a partir de estudos pré-clínicos com *D. melanogaster* expostas a drogas psicoativas, tais como, maconha, tabaco e cocaína. Foi utilizado o modelo de efeitos aleatórios de DerSimonian e Laird, usando o método de variância inversa para atribuir o peso dos estudos. A heterogeneidade estatística foi verificada pelo teste Q de Cochran e a inconsistência pela estatística I^2. A análise de sensibilidade foi realizada em caso de heterogeneidade significativa, remoção de um estudo por vez e análise de subgrupo. Para todas as análises, adotou-se um valor de alfa igual a 5%, utilizando-se o pacote metan do software Stata v13.0 (StataCorp, College Station, TX, EUA). A qualidade dos relatos escritos foi avaliada usando o CAMARADES adaptado. Cinquenta e um estudos preencheram os critérios de elegibilidade e foram usados para extração de dados. Desses, quinze foram elegíveis para a metanálise. A exposição à cocaína causa aumento da atividade locomotora em *D. melanogaster* (SMD, -1,11; IC 95% -1,81, -0,42). Doses baixas de nicotina causam aumento da atividade locomotora e doses altas causam diminuição. Embora não seja estatisticamente significativo, ainda há uma diminuição considerável na porcentagem de sobrevivência e aumento no tempo para 50% de eclosão após a exposição à nicotina. Além disso, também não significativo, é observado maior mudança de comportamento quando há exposição repetida à cocaína. Outros resultados foram discutidos qualitativamente. Esta análise fornece evidências claras de que drogas de abuso como cocaína, tabaco e maconha causam alterações moleculares, morfológicas, comportamentais e de sobrevivência em *D. melanogaster* em diferentes estágios de desenvolvimento, os quais são processos homólogos a humanos. Dessa forma, a coleta e análise dos dados descritos na literatura podem ajudar a compreender os impactos diretos na saúde humana.

Palavras-Chave: Drogas de abuso. *Drosophila*. Cocaína. Maconha. Nicotina.

1 INTRODUÇÃO

As drogas psicoativas são substâncias que, quando em contato com o organismo, interagem com o sistema nervoso central afetando os processos mentais, como a percepção, consciência, cognição ou humor e emoções (WORLD HEALTH ORGANIZATION, 2022). Cerca de 270 milhões de pessoas (5,5% da população global de 15 a 64 anos) usaram drogas psicoativas no último ano e estima-se que cerca de 35 milhões de pessoas sejam afetadas por transtornos por uso de drogas (WORLD HEALTH ORGANIZATION, 2022). Aproximadamente 0,5 milhão de mortes anuais estão relacionadas ao uso de drogas, com cerca de 350.000 mortes masculinas e 150.000 femininas (WORLD HEALTH ORGANIZATION, 2022). No Brasil, 4,9 milhões de pessoas (3,2% dos brasileiros) usaram substâncias ilícitas nos 12 meses anteriores a 2017. A droga ilícita mais consumida foi a maconha, seguida da cocaína em pó, depois o crack, LSD, medicamentos não prescritos, heroína, ecstasy, entre outros. Porém, os dados considerados mais alarmantes com relação aos padrões de uso de drogas no Brasil foram relacionados ao álcool (BASTOS, 2017). Com relação ao percentual de fumantes com 18 anos ou mais no Brasil é de 9,1%, representado por 11,8% dos homens e 6,7% das mulheres (INCA, 2022).

O uso de drogas psicoativas leva ao desenvolvimento de transtornos por uso de drogas, que compreende duas grandes condições de saúde: “padrão nocivo de uso de drogas” e “dependência de drogas” (GEORGE; KOOB, 2017; WORLD HEALTH ORGANIZATION, 2022). Os transtornos por uso de drogas aumentam os riscos de morbidade e mortalidade para os indivíduos (GLEA; PRESTON, 2020; WORLD HEALTH ORGANIZATION, 2022). Além disso, podem desencadear sofrimento substancial e levar a prejuízos nas funções pessoais, familiar, social, educacional, ocupacional ou em outras áreas importantes. Os transtornos por uso de drogas estão associados a custos significativos para a sociedade devido à perda de produtividade, mortalidade precoce, aumento dos gastos com saúde e custos relacionados à justiça criminal, bem-estar social e outras consequências sociais (MCLELLAN, 2017). São condições de saúde complexas desencadeadas por fatores determinantes como os psicossociais, ambientais e biológicos (GEORGE; KOOB, 2017) e se relacionam com alterações a nível molecular, neural e comportamental que são provocadas pelo uso da droga (ANDRETIC, 1999; BAINTON et al., 2000, 2005; GÓMEZ et al., 2019; HARDIE; ZHANG; HIRSH, 2007; LEE et al., 2010, 2011; PHILIPSEN et al., 2018; REN et al., 2012; VELAZQUEZ-ULLOA, 2017).

Nesse contexto, surge a necessidade dos estudos em animais, que têm sido cruciais para a compreensão da biologia e fisiopatologia da dependência de drogas e abuso de substâncias

(LYNCH et al., 2010). Em contraste com os estudos clínicos, os modelos animais podem ter variáveis controladas mais facilmente. Modelos animais permitem que o experimentador se concentre em componentes distintos do processo de dependência, variando de respostas simples e agudas a comportamentos mais complexos, como busca de drogas, autoadministração e recaída (LYNCH et al., 2010). Embora os modelos em roedores tenham fornecido informações cruciais sobre os mecanismos subjacentes aos comportamentos relacionados a drogas, eles não são ideais para abordagens genéticas avançadas destinadas a identificar mecanismos novos. Isso se deve principalmente ao gasto e tempo necessários para a manutenção dos animais, reprodução e análises comportamentais (KAUN; DEVINENI; HEBERLEIN, 2012). Em contraste, a mosca da fruta *D. melanogaster* é um dos organismos modelo mais geneticamente e experimentalmente acessíveis em biologia (KAUN; DEVINENI; HEBERLEIN, 2012). As vantagens clássicas do uso de *Drosophila* incluem fatores como custo, tamanho, fecundidade e escala de tempo (JENNINGS, 2011). E dessa forma, as moscas representam um organismo ideal para realizar triagens de mutagênese para isolar genes que regulam um determinado processo biológico de interesse (KAUN; DEVINENI; HEBERLEIN, 2012). Cerca de 75% dos genes de doenças humanas têm sequências relacionadas em *Drosophila,* sugerindo que as moscas podem servir como um modelo eficaz para estudar a função de uma ampla gama de genes envolvidos na doença humana (PANDEY; NICHOLS, 2011). Nos últimos anos, a geração de grandes coleções de mutantes publicamente disponíveis e outras ferramentas transgênicas permitiu o estudo funcional de quase todos os genes de interesse de moscas (ZIRIN et al., 2020; KAUN; DEVINENI; HEBERLEIN, 2012).

Apesar de todas as descobertas acerca do uso de drogas de abuso, ainda há lacunas a serem preenchidas, principalmente com relação a mecanismos moleculares e vias de sinalização mediando todas as alterações encontradas. Com isso, o nosso objetivo foi descrever como o tabaco, a maconha e a cocaína afetam o desenvolvimento normal de *D. melanogaster*.

ARTIGO 2

To be submitted to Critical Reviews in Toxicology

Effects of cocaine, nicotine and marijuana exposure in *Drosophila melanogaster* development: A systematic review and meta-analysis

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Abstract

Objective: The consumption of drugs of abuse is one of the main public health challenges leading to several adverse effects. The physiological effects of drugs of abuse are studied in animal models and Drosophila melanogaster stands out in this type of research. The main objective is to evaluate the changes caused by drugs of abuse in D. melanogaster, since this has been a well-studied model that may help to understand the underlying mechanisms of drug addiction in humans. **Methods:** We searched 7 online databases (PubMed, EMBASE, ISI Web of Science, LILACS, SIGLE, Science Direct, and Google Scholar), From the beginning of each database until January 2023, investigated Experimental studies with D. melanogaster exposed to Psychoactive drugs. Two reviewers independently extracted data. A systematic review and Meta-analyses were performed using the random-effects model of DerSimonian and Laird, using the inverse variance method to attribute the weight of the studies. Statistical heterogeneity was verified using Cochran's Q test and inconsistency using I^2 statistics. Sensitivity analysis was conducted in case of significant heterogeneity, removal of one study at a time, and subgroup analysis. For all analyzes, an alpha value equal to 5% was adopted, using the metan package of the Stata v13.0 software (StataCorp, College Station, TX, USA). The quality of the report was assessed using CAMARADES adapted. **Results.** Fifty-one studies met eligibility criteria and were used for data extraction, fifteen were eligible for meta-analysis. Exposure to cocaine causes increased locomotor activity in D. melanogaster (SMD, -1.11; IC 95% -1.81, –0.42). Low doses of nicotine cause an increase in locomotor activity and high doses cause a decrease. Although not statistically significant, still a remarkable decrease in the percentage of survival e increase in time to 50% eclosion following the exposure to nicotine. In addition, also not significant, there is a greater change in behavior when there is repeated exposure to cocaine. Other outcomes were discussed qualitatively. **Conclusion** This analysis provides clear evidence that drugs of abuse such as cocaine, tobacco and marijuana cause molecular, morphological, behavioral and survival changes in D. melanogaster at different stages of development.

KEYWORDS: Drugs of abuse. Drosophila. Cocaine. Marijuana. Nicotine.

1 INTRODUCTION

Psychoactive drugs stimulate the central nervous system (CNS) and can be classified as hallucinogenic, depressive, and psychostimulant (Chen and Lin 2009; Ayala 2009; Gatch et al. 2016). The use of psychoactive substances is as old as human history, being strongly associated with religious practices, medicinal actions, recreation, and collective rituals (Crocq 2007; Goldstein et al. 2009; Kosiba et al. 2019). The continuous and progressive use of drugs, such as cocaine, tobacco/nicotine, and marijuana, may trigger several molecular, neural, and behavioral changes, which can lead to addiction (Andretic 1999; Bainton et al. 2000; Bainton et al. 2005; Hardie et al. 2007; Lee et al. 2010; Lee et al. 2011; Ren et al. 2012a; Velazquez-Ulloa 2017; Philipsen et al. 2018; Gómez et al. 2019).

Tobacco exposure has been associated with cancers and cellular alterations, chronic diseases (especially pulmonary, cardiovascular and endocrine), as well as the development of oral, tissue and/or molecular pathologies, gestational alterations and cognitive effects) (le Foll and Goldberg 2009; Wagenknecht et al. 2018). While cocaine use can lead to cardiovascular and neurophysiological (i.e. generation of seizures) changes; maternal, fetal, and neonatal consequences (Koppel et al. 1996; Sordo et al. 2013; Chang et al. 2016; dos Santos et al. 2018; Pacheco et al. 2021); brain stroke (Levine et al. 1990; Neiman et al. 2000; Merigian et al.); as well as behavioral disorders, such as changes in locomotion, and emotional, cognitive, sexual and hormonal responses (Bainton et al. 2000; Matsuo et al. 2013; Filošević et al. 2018; Shibuya et al. 2021). Finally, tetrahydrocannabinol (THC), the main psychoactive substance in marijuana, has been linked to schizophrenia and several types of cancers (Nahas and Latour 1992; Laviola et al. 1999). However, many beneficial effects of cannabidiol, another substance present in marijuana, have been discussed in several studies regarding the treatment of chronic pain and refractory epilepsies (Rodríguez-Muñoz et al. 2018; Upadhya et al. 2018; Crivelaro do Nascimento et al. 2020).

Currently, researchers have focused, especially, on the study of drug abuse and its consequences. The compulsive use of drugs characterizes the state of addiction continuously rising in the population, resulting in physical and social damages that lead to serious public health problems (Ghezzi and Atkinson 2011; Wilkinson et al. 2016; Holbrook 2016; Kampman 2019; Ryan 2019). Despite the growing impact on public health, the pathophysiological mechanisms are not completely understood.

Based on this, the use of Drosophila melanogaster as an animal model in neuroscience has become widespread, especially in the area of drugs of abuse. The main reason is a significant similarity with vertebrates in the main neurotransmitter systems involved in the

response to psychostimulants, such as the endocannabinoid dopaminergic and cholinergic systems (McPartland et al. 2006; Matsuo et al. 2013; Velazquez-Ulloa 2017). The discovery of these similarities allowed the use of D. melanogaster as a promising animal model in the study of behavioral, physiological, neurochemical, neuroanatomical and genetic characteristics resulting from exposure to drugs of abuse, as well as helping to discover new molecular targets and identifying related toxicological and/or pathological changes (Bainton et al. 2000; Maia et al. 2007; Heberlein et al. 2009; Ghezzi and Atkinson 2011; Pandey and Nichols 2011; Kaun et al. 2012; Yamamoto and Seto 2014; de Nobrega and Lyons 2017; Filošević et al. 2018; Chen and Read 2019; Shibuya et al. 2021).

Therefore, the purpose of this systematic review was to identify the main changes in the behavior and development of D. melanogaster exposed to cocaine, tobacco/nicotine, and marijuana. In this way, the gathering and analysis of data described in the literature could help to understand the direct impacts on human health.

2 METHODS

This systematic review was based on the guideline of Preferred Reporting Items for Systematic Reviews and Meta-analyzes (PRISMA) (Page et al. 2021). The protocols were registered on the International Prospective Register of Systematic Reviews (PROSPERO; nº. CRD42020146823), and it is available at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020146823.

2.1 Eligibility criteria

The research strategy aimed to identify the main changes in the behavior and development of D. melanogaster exposed to psychoactive drugs cocaine, nicotine and marijuana. Articles published from the starting date of each database to January 2023 were included. No language restriction was applied.

The articles that met all the following established criteria were included: i. the relevant intervention group for this review was D. melanogaster, which was exposed to psychoactive drugs (tobacco, nicotine, marijuana, and cocaine), including their metabolites; ii. experimental models using D. melanogaster or mutant strains regardless of gender or development stage; Only original research from preclinical experimental studies; iii. the control group was considered as flies that were not exposed to abuse drugs; iv. there was no restriction for the duration, route of administration or dose and published from the starting date of each database to January 2023.

2.2 Identification of relevant studies

Databases searched included PubMed (from 1960 to January 2023), EMBASE (from 1947 to January 2023), ISI Web of Science (from 1900 to January 2023), LILACS (from 1982 to January 2023), SIGLE (from 2007 to January 2023), Science Direct (from 1997 to January January 2023), and Google Scholar (from 2004 to January 2023). A search strategy was carried out using a combination of base descriptors "Drosophila melanogaster" or "fruit flies", and "cocaine", "marijuana", "tobacco" or "nicotine" (MeSH). We don't use filters. The search strategies are shown in Table 1.

Table 1 Search strategies for each database

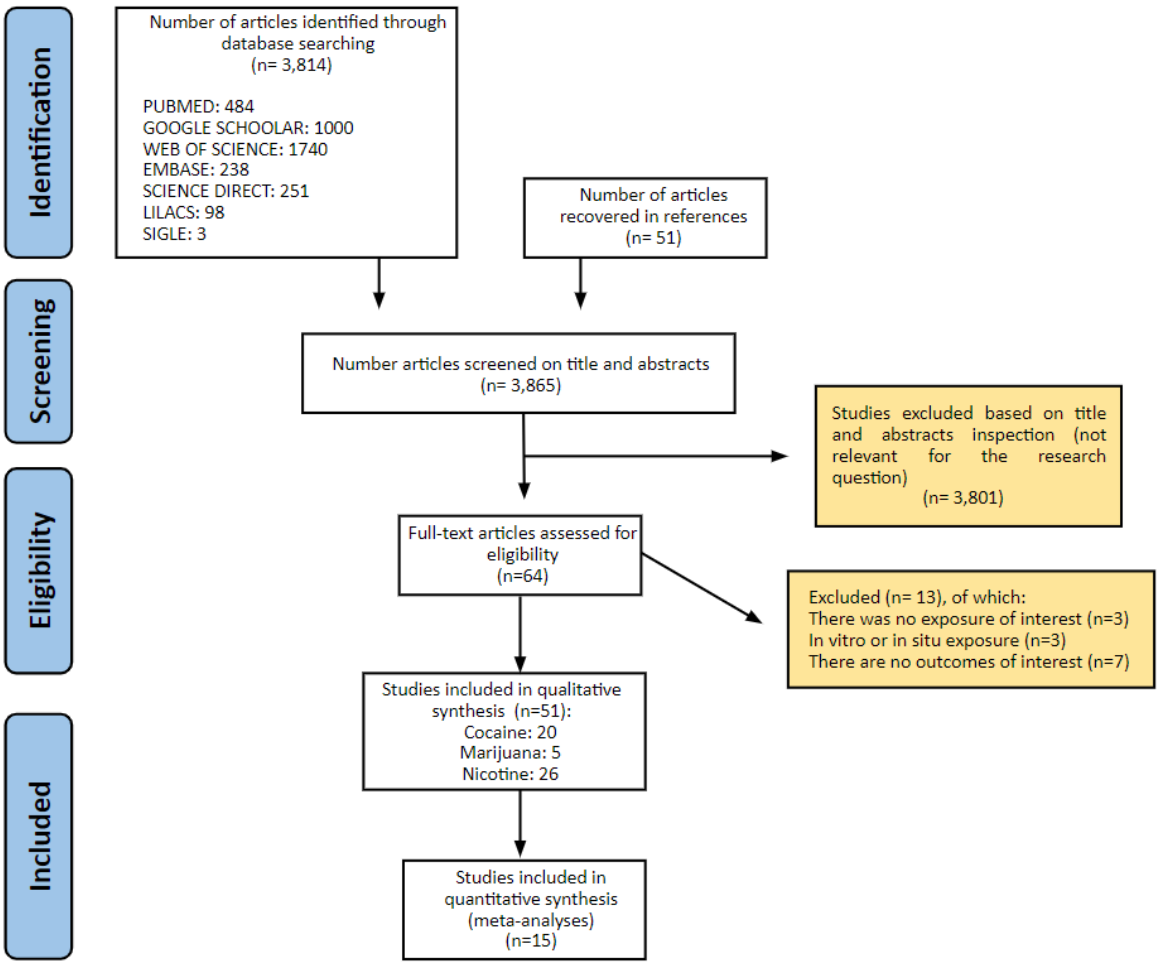
PUBMED	(("fruit"[MeSH Terms] OR "fruit"[All Fields]) AND ("diptera"[MeSH Terms] OR "diptera"[All Fields] OR "flies"[All Fields]) OR "drosophila"[MeSH Terms] OR "drosophila"[All Fields] OR "drosophila melanogaster"[MeSH Terms] OR ("drosophila"[All Fields] AND "melanogaster"[All Fields]) OR "drosophila melanogaster"[All Fields]) AND ("illicit drugs"[MeSH Terms] OR ("illicit"[All Fields] AND "drugs"[All Fields]) OR "illicit drugs"[All Fields] OR ("street"[All Fields] AND "drugs"[All Fields]) OR "street drugs"[All Fields] OR "cocaine"[MeSH Terms] OR "cocaine"[All Fields] OR "tobacco"[MeSH Terms] OR "tobacco"[All Fields] OR "tobacco products"[MeSH Terms] OR ("tobacco"[All Fields] AND "products"[All Fields]) OR "tobacco products"[All Fields] OR "nicotine"[MeSH Terms] OR "nicotine"[All Fields] OR "cannabis"[MeSH Terms] OR "cannabis"[All Fields] OR "marijuana"[All Fields])
SIGLE	(Fruit flies OR drosophila OR drosophila melanogaster) AND (street drugs OR cocaine OR marijuana OR tobacco)
EMBASE	('Fruit flies'/exp OR 'drosophila'/exp OR 'drosophila melanogaster'/exp) AND ('street drugs'/exp OR 'cocaine'/exp OR 'marijuana'/exp OR 'tobacco'/exp OR 'nicotine'/exp)
WEB OF SCIENCE	(Fruit flies OR drosophila OR drosophila melanogaster) AND (street drugs OR cocaine OR marijuana OR tobacco OR nicotine)
LILACS	(mj:(drosophila)) AND (mj:(cocaine)) OR (mj:(nicotine)) OR (mj:(tobacco)) OR (mj:(marijuana)) OR (mj:(street drugs))
GOOGLE SCHOLAR	drosophila melanogaster "drugs of abuse"
Science Direct	(("drosophila melanogaster") and ("drugs of abuse"))

Initially, two independent investigators (J.F.S. and I.R.R.B.) conducted the searches, comparing their results. All titles and abstracts were read by the reviewers independently and, where appropriate, full-text articles. A third reviewer (I.S.M.) was consulted when there was no consensus on the inclusion of data or a particular article. Subsequently, the reviewers read full-length manuscripts and reached a consensus about their inclusion. We included in this systematic review articles that met all the established criteria but did not present standard data for meta-analysis. It was not possible to assess the quality of the studies, but we assessed the quality of the report. Comments, letters, editorials, and reviews were excluded. To find other eligible studies, the references of included articles were checked.

2.3 Data extraction

The initial data extraction was carried out by the same reviewers independently. The excluded articles were considered and the reason for their exclusion was indicated (Fig. 1). Reviewers discussed disagreements at consensus meetings. The general characteristics of the selected studies were properly collected, including the authors, year of publication, fly strain, gender, stage of development, number of flies, exposure (type, dose, route, and frequency) and the main reported results.

Figura 1. Literature search flowchart



A series of predetermined variables were extracted from each included paper. The

primary outcomes of interest were survival, mortality, pupariation, ET50 (number of days required for 50% of flies to hatch), malformation, weight (mg), locomotor activity, cerebral hemisphere area (mm2), number of positive neurons for tyrosine hydroxylase, time to 50% sedation (ST50), climbing agility, ability to fly (%), enzyme activity (catalase, SOD, Glutathione – (unit/mg protein)), behavior alterations, fat body content (µg/µg/fly), differential gene expression, percentage of impaired flies, cardiac function (transient calcium), reactive oxygen species (DCF µmol), oxidative stress, number of eclosed flies.

2.4 Data analysis

For Metanalise we used the graphical application WebPlotDigitizer (version 4.6) to facilitate easy and accurate data extraction (Ankit Rohatgi 2022).

For continuous outcomes that present their data in means and are measured in the same unit of measurement, the weighted mean difference between treatments was calculated, associated with their 95% Confidence Interval. For outcomes that are measured using different scales/metrics, the standardized mean difference between treatments was calculated, also with the 95% confidence interval. Finally, for outcomes that are presented as frequencies, the difference in risk between treatments was calculated. All calculations followed the random-effects model of DerSimonian and Laird, using the inverse variance method to attribute the weight of the studies.

Statistical heterogeneity was verified using Cochran's Q test and inconsistency using I^2 statistics. Sensitivity analysis was conducted in case of significant heterogeneity, removal of one study at a time, and subgroup analysis. For all analyzes, an alpha value equal to 5% was adopted, using the metan package of the Stata v13.0 software (StataCorp, College Station, TX, USA).

2.5 Quality of the report assessment

We had planned to use the SYRCLE risk of bias tool for animal studies (Hooijmans et al. 2014). However, we have observed that studies using the D. Melanogaster animal model have specificities that SYRCLE cannot address. Perhaps this is the reason why systematic reviews have not used any tools to assess the risk of bias.

The quality of the report assessment process of the studies in this systematic review needed to be adapted to the characteristics of the intervention studies with D. melanogaster. The CAMARADES 10-item study quality checklist was used to assess the quality of the report

articles. This checklist was modified based on the models of D. melanogaster exposed to drugs of abuse as (1) peer-reviewed publication, (2) control of environmental conditions (temperature, food, light/dark cycle), (3) description of the strain in the control and exposed groups, (4) description of age and sex (5) frequency and duration of exposure to drugs of abuse, (6) stage of flies exposed to drugs of abuse, (7) dose of abuse drugs for exposure, (8) control group for comparison, (9) sample number, (10) statement of potential conflict of interests (Macleod et al. 2004; Zeng et al. 2015).

3 RESULTS

3.1 Qualitative description of included studies

As a result of the initial search of the database, a total of 3814 studies were identified and 51 were recovered from the references. After selecting and analyzing the 3865 articles based on titles and abstracts, 3801 studies were excluded because they were not relevant to the research question. 64 full-text articles were assessed for eligibility, of which 13 were excluded. The reasons for exclusion from the studies involved the absence of exposure (n = 3) or outcomes (n = 7) of interest, as well as in vitro or in situ exposure (n = 3). Then, 51 studies met inclusion criteria and were included in this systematic review, addressing the different drugs of abuse: nicotine (n = 26), cocaine (n = 20), and marijuana (n = 5). Finally, only 15 articles were included in the meta-analysis (Figure 1). The main characteristics of the 51 included studies are shown in Table 2. All these articles were published in English between 1998 and 2022 and were preclinical experimental studies with D. melanogaster. Most studies (n = 24) used male flies, distributed in cocaine (n = 14), nicotine (n = 7) and marijuana (n = 3). While only 15 used both sexes, distributed in cocaine (n = 5), nicotine (n = 9) and marijuana (n = 1), some nicotine studies used larvae (n = 5) and one study did not report sex. Among the 51 articles included, 37 used adult flies, 8 used larvae, and 6 used both developmental stages. The exposure to different drugs of abuse (nicotine, cocaine, and marijuana) was performed through different routes, including intraperitoneal, injected, volatilized, smoked or by feeding the flies. In addition, the frequency of exposure to these drugs of abuse ranged from minutes to days. The main outcomes of exposure to these drugs of abuse are shown below (Table 3).

Table 2. Characteristics of studies selected for systematic review.

First author and study year	Quality score	Strains of flies	Gender	Developmental stage of the flies	Number of flies	Exposure			Outcomes reported
						Type and dose	Route	Frequency	
Cocaine exposure in Drosophila melanogaster									
McClung and Hirsh, 1998	7	Oregon R	Male and Female	Adult	20 - 80	Cocaine (75 µg, 100 µg and 110 µg)	Volatilized	once for 1 min or three times, (on day 1) or two times (on the following day) with 3 hour intervals between doses in both	behaviors elicited by cocaine: Males are more severely affected than females and develop a significant sensitization to cocaine by the third exposure, which continues through the following day.; the flies progress rapidly to their most severe behavioral response and then display the behaviors in descending order during the recovery period; sensitization in both sexes, with maximal sensitization occurring with a time interval of 6 hours between doses.
Torres *et al.*, 1998	9	Wild-type (Canton-S strain)	Female and male	Adult	10 - 32	Cocaine (1mM)	Injected	Exposed for 1 min	↑ behavioral activity
McClung and Hirsh, 1999	8	Wild-type (Oregon R) or Drosophila mutant inactive (iav), $T\beta H^{M18}$	Male	Adult	54 - 80	Cocaine (75 µg)	volatilized cocaine free base	two exposures at a 6-h interval or 10 exposures of	Cocaine: ↑ in the number of flies showing more severe behaviors with scores ≥ 5 following the second cocaine dose.

								over 5 days for TDC activity	Defective sensitization in iav flies, but near-normal sensitization in the TβHM18 null mutant. Cocaine-sensitized flies TDC activity was elevated approximately 80% compared with sham-treated controls
Andretic et al., 1999	7	WT Oregon^{R} and *per*^{0}	Male	Adult	30 – 50	Cocaine (75µg)	Volatilized free-base	3 times over 2 days	WT flies: ↑ locomotion after exposure per0 flies: no sensitization to cocaine
Bainton et. al., 2000	8	WT Canton-S	Male	Adult	9 – 10	Cocaine (0, 100, 200, 300, 400 and 500µg)	Volatilized (dissolution in ethanol)	Exposed for 1 min	Exposed WT flies: ↓ climbing
Park et.al., 2000	7	WT W^{1118} and pka-RII$^{EP(2)2162}$	Male	Adult	48 -78	Cocaine (75, 90,150 and 200µg)	volatilized free-base cocaine	two exposures at a 6-h interval	subsequent doses of cocaine failed to elicit robust sensitization in pka-RII$^{EP(2)2162}$ flies, whereas wild type flies show prominent sensitization, measured as an enhanced response to the second cocaine exposure.
Li et. al., 2000	8	wild-type (w^{1118}), pDdc–GAL4, pDdc–GAL4.3D and pDdc–GAL4.36, UAS–TNT-E, UAS–Gαi, and UAS–Gαs.	Male	Adult	15 - 125	Cocaine (40, 60, 75 e 90 µg) or 0.05 mg/ml cocaine–HCl	volatilized free base or applied to the nerve cords	once or three cocaine doses at 0, 6 and 24 h	Cocaine: ↑ hypersensitivity and locomotor responses em $G\alpha_i$ or TNT; ↓ hypersensitivity and locomotor responses in Gαs compared with controls; Repeated exposures led to robust sensitization in controls,

									but $G\alpha_i$, TNT or $G\alpha s$ abolished sensitization.
Dimitrijevic et al., 2004	9	WT Canton S and *per*^{0}	Male	Adult	22 – 24	Cocaine (20, 100 or 200 pmol per fly)	Injected	Once or twice	WT and *per*^{0} flies: ↑ locomotor activity and number of "peaks" after cocaine (100 and 200 pmol) Repeated cocaine injections: ↑ behavioral responses in WT but not in *per*^{0} flies
Tsai et al., 2004	10	Wild-type melanogaster flies	Male	Adult	15	Cocaine (75, 100, 150, 200 and 250µg)	Volatilized cocaine	Once	EP1306 flies show changes in locomotor patterns induced by cocaine from activity.
Bainton et al., 2005	8	EP369 (WT control), EP1529 Moody-α (α1 and α2) and Moody-β (β1 and β2) in D*17* flies	Male	Adult	12 – 20	Cocaine (100, 200 and 400µg)	Volatilized (dissolution in ethanol)	Exposed for 1 min	EP1529 flies: ↑ cocaine sensitivity Independent moody-α (α1 and α2) and moody-β (β1 and β2) in D17 flies: cocaine sensitivity did not restore
Lease and Hirsh, 2005	9	Oregon R (OreR)	Male	Adult	81	Cocaine	airbrush (1, 2, 3 and 4 µg/µl) or volatilized or volatilized with pre-drug (50, 60, 70, 80, 90 and 100 µg) exposure cold treatment.	A 1.5 s spray followed by 10 s of air alone to dose and then dry the flies.	↑ The stereotypic responses are dose-dependent in the airbrush and smoke cocaine. The variation in behaviors of flies dosed with the airbrush was smaller than that of the smoke-dosed flies, indicating that the airbrush method gives better reproducibility.

Chang et al., 2006	8	WT Oregon^{R} and transgenic flies (DVMAT)	Male and female	Adult	20 – 36	Cocaine (0.1, 1 or 10µg/mL; 200µg)	Feeding or volatilized cocaine	5 or 7 days	WT flies: ↑ locomotion and grooming after exposure DVMAT overexpression: ↓ sensitivity to cocaine and impairment in climbing
Sedore Willard et al., 2006	9	Oregon-R, Canton-S and Dahomey (DH)	Female	Adult	4 - 141	Cocaine (0.75, 1.0, 1.5, 2.0, 2.25 mg/ml)	Food	during adult lifespan.or treatments of 10 days or longer	Cocaine: ↓ adult lifespan and results in morphological defects in ovarian follicles.
Hardie et al., 2007	8	Cantonized w^{1118} [CS] and *Tdc1* transgene	Male	Adult	90	Cocaine (85 µg)	Volatilized free-base	Once	Cantonized w^{1118} [CS] flies: ↑ locomotor activity Tdc2^{RO54} flies: ↓ basal locomotor activity levels and are hypersensitive to an initial dose of cocaine.
Lebestky et. al., 2009	9	CS (+/+) flies; black bars, DopR/DopR flies.	Male	Adult	10	Cocaine (50 0 and 750 µg)	Food	for the 48 hr incubation, eating ad libitum.	↓ of τ and distance traveled by cocaine in CS flies and the lack of an effect of cocaine in DopR/DopR flies
Gakamsky et. al., 2013	9	Canton-S (CS)	Male	Adult	8	Cocaine (120 µg)	volatilized	During the experiment	Cocaine: ↑ angular interval
Filoševic et al., 2018	9	WT Canton S and mutant for circadian genes (*Clk*^{Jrk},	Male and female	Adult	32	Cocaine (25, 50, 75, 100, 125 or 150µg)	Volatilized (dissolution in ethanol)	Twice or 3 times for 1 min	WT flies: ↑ locomotor activity in the first minute after cocaine (25-150 µg)

		per^{01}, *cyc*^{01}, *tim*^{01} and *pdf*^{01})							*per*, *Clk* and *cyc* genes: required for locomotor sensitization to cocaine
Philipsen et. al., 2018	8	Canton-S	Male	Adult	5 - 11	cocaine (15 mM)	food supplemented with cocaine	3 days	Cocaine administration: Increased Phospholipid species, particularly phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and phosphatidylinositols (PIs), in the central brain and the optical lobes in Drosophila brain.
Philipsen et. al., 2020	10	Canton-S	Male	Adult	24 - 33	cocaine (15 mM)	food supplemented with cocaine	3 days	Cocaine increase the levels of phosphatidylcholines in the fly brain; after drug withdrawal, the abundance of these lipids returns to the original level and methylphenidate treatment of the flies following cocaine exposure enhances the reversal of the lipid level reducing them below the original control.
Baker et. al., 2021	10	Canton-S	Male and Female	Adult	114 - 166	Cocaine (0.53 μL)	CAFE Assay	no more than 2 h	Male flies exposed to cocaine took longer to climb in the negative geotaxis assay than control flies, whereas females appeared unaffected excessive grooming behavior in a fraction of male flies exposed to cocaine.

									seizures in a small percentage of flies after cocaine intake during the negative geotaxis assay. Seizures rarely occurred in controls . Collectively, these experiments provide evidence that acute exposure to cocaine results in neurological impairments. Annotation of clusters based on gene markers revealed that all major cell types (neuronal and glial) as well as neurotransmitter types from most brain regions were represented.
Nicotine exposure in Drosophila melanogaster									
Carrillo and Gibson, 2002	7	Parental lines used in this study consisted of 16 iso-female lines of *D. melanogaster*.	Male and Female	Adult	10	Nicotine (3 **µ**l/mL)	Nicotine food	Adulthood, until all of the flies were dead	In general, females are twice as resistant to nicotine as males
Hou et. al., 2004	7	Canton-S, OGS-4, *dnc*^{1}, *dnc*^{M11}, y w; DC0^{B3}/CyO P{y^{+}}, DC0^{H2}/CyO, S30 e S64.	Male	Adult	10 - 25	Nicotine (2 µL) - 0, 20, 50, 100, 200 and 400 µg of nicotine in CS flies.	Volatilized	for 1 min in some cases for four times	<u>Startle-induced climbing response:</u> Nicotine Canton-S - Response inhibited in a dose-dependent manner; *dnc*^{1} and *dnc*^{M11} - was depressed by nicotine significantly more strongly than in wild type flies;

									$DC0^{H2}$ and $DC0^{B3}$, showed low sensitivity to nicotine Spontaneous locomotor activities: transiently ↑ (nicotine at 50 µg), but tended to be ↓ by higher doses of nicotine. In *dnc*^{1} and $DC0^{B3}$ were significantly ↓ (nicotine 100 µg and 200 µg).
Passador-Gurgel et. al., 2007	9	North Carolina and California populations	Female	Adult	50–60 flies per vial	Nicotine (Sigma), 3 µl/ml.	Nicotine food	8 h of chronic exposure to the nicotine food	Nicotine: shows no relation to survival time. However, the longer survival time reflects for the North Carolina population 15 (Carolina) and 11 (Northern and California) genes, respectively, showed level of regression of abundance of transcripts in survival time
Gui and Grant 2008	8	D. melanogaster wild-type strain	not applicable	Larvae	40	0, 60, 120, and 180 µg/ml nicotine	nicotine food	Larval phase	High nicotine concentration: ↓pupation Intermediate concentrations: ↑ pupation Nicotine (120 µg/mL): ↑ survival
Hamatake et al., 2009	7	WT strain (Oregon-R) and a urate-null strain (y v ma-l)	Male and female	Larvae and Adult	3 – 141	Three burning cigarettes.	smoke	20 min during exposures for 3 or 6 h.	urate-null strain (y v ma-l): ↓The survival after 6-h ESC exposure.
Fujiwara et al., 2011	7	WT strain (Oregon-R, Hikone-R and Canton-S) and	not applicable	Larvae	500	burning cigarettes	smoke	20 min during exposures for 2, 4 or 6 h.	The survival ↓ wild-type strains Oregon-R and Hikone-R (6 h), Canton-S

		a urate-null strain (y v ma-l)							(3 h), and 3 to 4 h for the urate-null mutant strains. The urate level: ↑ In both wild type strains, unlike the case in either of the mutant strains, in a manner dependent on the duration of ECS exposure. glutathione level: ↑ Canton-S and in both of the urate-null strains
Li et. al., 2012	9	D. melanogaster natural populations collected from California (CM1, CM2, CM3, and CM7) and Africa (AM2, AM3, AM4, AM7).	Male and female	Adult	30 - 222	0.5, 1, 2, or 4 mg/g nicotine sulfate	Nicotine-containing food	2–8 days post eclosion	The four strains with Accord-mediated CYP6G1 overexpression are resistant to nicotine.
Ren et al., 2012	10	WT Canton-S, w^{1118}, V1194 and mutante (mutant Dcp2V1194/+, Dcp2c02419/+, and Dcp2e00034/+) flies	Male	Adult	28 – 32	Nicotine (0.6, 1.8, 3.0, or 4.2 mM)	Nicotine-containing food	3 or 4 days	CS flies: ↑ locomotor activity after nicotine (3mM); w1118 flies: ↑ locomotor activity after nicotine (3mM); V1194 flies: ↓ locomotor activity after nicotine (3mM); Heterozygous mutante Dcp2V1194/+, Dcp2c02419/+, and Dcp2e00034/+ flies show chronic nicotine-induced locomotor hyperactivity

Chambers et. al., 2013	9	park$^{25/25}$, w^{1118}	Male and Female	Adult	10 - 20	Nicotine (0, 9 or 12 µg/ml)	Nicotine-containing food	From day one post eclosion, Flies were tested on days 5, 10, 15 and 20 post eclosion.	Nicotine: (9 or 12 µg/mL) ↓ LT_{50} in control flies (w^{1118}); (9 µg/mL) ↑ LT_{50} in park^{25} heterozygous flies. Nicotine: improves climbing ability and the progressive decline in flight behavior in park25 heterozygotes in Heterozygous mutation
Fuenzalida -Uribe et. al., 2013	9	Wild-type Canton-S, Tdc2-Gal4, DDC-Gal4, UAS-RNAiTbH, UAS-RNAiDDC, UAS-TeTx.LC	Male	Adult	10	Nicotine (6 µg)	volatilized	for 1 min	Nicotine: ↓ startle response in genetic control flies (Cs/RNAiTbH). There were no changes in the flies that express an RNAi for tyramine beta-hydroxylase (TbH) (Tdc2/RNAiTbH), flies that express a mutation for the TbH enzyme (tbHmut) and flies that express the tetanus toxin in octopaminergic neurons have no induced effect nicotine (Tdc2/Tetx).
Hill-Burns et. al., 2013	10	w^{1118}	Female	Adult	30 flies for vial	0, 0.01, 0.05, 0.1, 0.2, 0.4 mg/ml nicotine	Nicotine-containing food	8 – 10 days	Nicotine: improved survival for paraquat-treated flies in a dose-dependent manner. However, at high dose (0.4 mg/ml), became toxic for flies that were not exposed to paraquat causing decline in median survival.

									CG14691 gene expression was increased significantly in response to paraquat and restored to normal with co-treatment with nicotine.
Sadiq and Altaany, 2013	10	WT Oregon-K	Male	Adult	100 – 175	Cigarette smoke filtrate (SF; 0.05, 1.0, 2.0, 2.5, 0.5, 1.5 and 2.5%)	Intraperitoneal	Once	Survived males fliesat the 0.05%, 2.5% or 10% SF: 63%, 16% and none, respectively.
Marriage et. al, 2014	9	Drosophila Synthetic Population Resource	not applicable	larvae	30	0.18 µl/ml nicotine	nicotine food	larval phase	68.4% of the broad-sense heritability for nicotine resistance Ugt86Dd, Cyp28d1 and Cyp28d2 explain a large fraction of the genetic variation in larval resistance to nicotine.
Sanchez-Díaz et. al., 2015	10	w^{1118} and Ore-R (controls) and L70 and L4 mutations	Male	Adult	100	Nicotine (0.5 mg/ml)	Standard cornmeal food, supplemented with nicotine	3 times	L70 and L4 insertion lines: sensitive to chronic nicotine exposure. Mutant genotypes: ↓ lifespan when maintained in standard cornmeal food, supplemented with nicotine.
Uchiyama et al., 2015	7	WT Oregon-R and mutante (urate-null strain) flies	Male and female	Third instar F1 larvae and adult	unreported	Cigarette smoke	Smoke	Exposed to CS for 2, 4 or 6 h	Urate-null females (ma-l (−/−)): ↑ number of mutant spots in an exposure time-dependent, but not in the urate-positive females (ma-l (+/−)).
Zhang et. al., 2016	9	w1118 and Canton-S (CS)	Male and female	Adult	50–100	Nicotine (0.6 mM)	Nicotine was added to the normal food	unreported	w^{1118} and Canton-S: ↑ locomotor activity after

									nicotine in both males and females.
Highfill et. al., 2017	8	Drosophila Synthetic Population Resource	not applicable	Larvae	30	0.18 μl/ml nicotine	Nicotine food	Larval Phase	RNAseq showed that Ugt86Dd had significantly higher expression in genotypes that are more resistant to nicotine
Velazquez-Ulloa, 2017	9	w^{1118} Berlin (wB)	Male	Adult	50–100	Nicotine (0, 0.1, 0.2, 0.3 or 0.4 mg/ml)	Nicotine-laced food	From egg to adult	w^{1118} Berlin flies: ↓ Percent survival and ↑ time to 50% eclosion (ET50) with increasing nicotine concentrations (0.2, 0.3 and 0.4).
Prange et. al., 2018	8	Canton S, w^{1118} strain and larvae carrying upd-Gal4, UAS-GFP, upd2-Gal4, UAS-GFP, upd3-Gal4 or UAS-GFP.	Male and female	Adult and larvae	10 flies per vial	cigarette smoking	Smoke	30 min each day on 5 day per week, for 7, 10 or 14 days.	Chronic cigarette exposure to smoke: ↓ the survival, ↓ locomotor activity, ↓ reduced body fat, ↑ metabolic rate and ↓ in respiratory surface. TGF-β, Nrf2 and the JAK / STAT signaling pathways are altered by chronic exposure to cigarette smoke.
Morris et. al., 2018	10	w^{1118} Berlin (wB) strain	Male and female (when adults)	Larvae	15 - 95	0.3 or 0.1 mg/ml nicotine	nicotine food	larval phase	Nicotine: ↓ The number of eclosed flies and ↑ The number of days needed for 50% of the flies to eclose ↑ Larval brain hemisphere área ↓ Corrected TH central brain fluorescence Did not affect the number of TH+ neurons at the larval stage

									Had a cluster-specific effect in the adult dopaminergic system of the Drosophila brain.
Macdonald and Highfill, 2020	8	Drosophila Synthetic Population Resource	not applicable	Larvae	30	0.18 µl/ml nicotine	nicotine food	larval phase	The naturally-occurring 22-bp insertion/deletion event in Ugt86Dd directly impacts variation in nicotine resistance in D. melanogaster.
Santalla et. al., 2021	9	TinC- Gal4-UAS-GCaMP3	unreported	Adult and Semi-intact preparation	4 - 14	commercial cigarette smoke (2 doses of 10 cc, composition in mg for each cigarette: 10 tar, 0.7 nicotine and 10 carbon monoxide) in Semi-intact preparation (1.69 mM of nicotine).	cigarette smoke	daily for 7 days, 5 minutes each time at an interval of 6 hours	Commercial cigarette smoke affects cardiac performance of adult Drosophila. Acute administration of nicotine mimics some of the cigarette effects.
Carvajal-Oliveros et. al., 2021	10	Sph-1 expressing fies, α-synuclein (SNCA) expressing fies	Male	Adult	100 - 200	nicotine (24 µM)	Food	third day for the duration of the experiment.	Nicotine treatment increases lifespan in Sph-1 expressing fies, while reduces it in control animals.Flies expressing SNCA in dopaminergic neurons have a signifcantly reduced life expectancy which is

									similar in chronic nicotine treated animals (24 μM).
El-Merhie et. al., 2021	10	Canton S	Virgin female flies and the F1-generation	Adult and larvae	10 - 100	electronic nicotine delivery system (1, 5 or 10 mM or 8 μg)	nicotine vapor	once per hour for a total of eight times.	e-nicotine not only leads to reduced maternal fertility, but also negatively affects size and weight, as well as tracheal development of the F1-generation, lasting from embryonic stage until adulthood.
Mannett et. al., 2022	10	park[25] and w1118	Male and Female	Newly eclosed flies	21 - 56	Nicotine (10mg/mL)	food	Flies were transferred every 2-3 days onto new vials, with or without nicotine, until the day of the assay.	Nicotine Improves Climbing Deficits in Homozygous park25 Flies When Given on Day 0 and Improves Flight Ability in Homozygous park25 Flies.
Sirocko et. al., 2022	10	wildtype strain CantonS	Male and Female	Larvae	n = 4 independent experiments (each experiment: whole tissue from larvae or airways from 40 to 50 larvae)	Mainstream cigarette smoke (CS) 4 puffs/min	smoke	4 puffs/min for a duration of 60 min.	Taken together, early-life CS induces airway epithelial stress responses and dysregulates pathways involved in the fly's branching morphogenesis as well as in mammalian lung development. CS further affected fitness and development in a highly sex-specific manner.

Lee et. al., 2010	8	Canton S	Male	Adult and larvae	15 - 100	hempseed meal	food	embryos unt il they matured into adult flies	HSM: Increased body weights; shorter pupariation times; Survival were unaltered; increased concentration of sterol and reduced the levels of triglycerides
Lee at. al., 2011	7	wild-type strain, Dpark[1]	Male	Adult and larvae	10- 200	hempseed meal	food	Adult phase / larvae phase	HSM: increases the service life when exposed to H2O2, suggesting antioxidant action; The climbing ability of parkin mutant flies were unaffected; Reduction of cholesterol uptake by HSM feeding
Gómez et. al., 2019	10	Canton-S, handC-GFP, GCaMP3 (genotype: UAS-GCaMP3/+; tinCΔ4-Gal4, UAS-GCaMP3/+)	Male and famale	Adult and semi-intact heart preparations	7 - 196	0.03 g of dried herbal cannabis	vaporized	two daily doses and remained in contact with the vaporized substances for 15 min each for 6–8 or 11–13 days.	Consumption of cannabinoids along the entire life did not affect lifespan of flies marijuana: Arrhythmicity index is incremented during short-term exposure; Contractility is significantly incremented in flies treated for 11–13 days; Heart rate remains unchanged. Cannabis increments Ca^{2+} transient amplitude but does not modify SR calcium load, fractional release, SERCA and NCX activity in the Drosophila heart.
Yejin Ahn et. al., 2021	10	Canton-S	Male	Adult	10	hemp seed ethanol extract (0.5,	Food	7 days	The behavioral patterns of individual flies were significantly reduced with

						1.0, and 1.5%)			0.1% CPZ treatment. In contrast, combination treatment of 1.5% HE and 0.1% CPZ significantly increased subjective daytime activity and behavioral factors. These results correlate with increased transcript levels of dopamine and serotonin receptors and concentration of dopamine, levodopa, 5-HTP, and serotonin compared to those in the control group.
Vitorovi´c et. al., 2021	9	Oregon-R-C strain	-	Larvae	25 - 100	hemp seed oil (12.5, 18.7, 31.2, 62.5, and 125 µL/mL)	Food	72h or on the life cycle of D. melanogaster	The results revealed that under non-stress conditions, oil concentrations up to 62.5 µL/mL did not induce negative effects on the life cycle of D. melanogaster and maintained the redox status of the larval cells at similar levels to the control level. Under oxidative stress conditions, biochemical parameters were significantly affected and only two oil concentrations, 18.7 and 31.2 µL/mL, provided protection against hydrogen peroxide stress effects. A higher oil concentration (125 µL/mL) exerted negative effects on the oxidative status and increased larval mortality.

WT = Wild-type; Hempseed meal media (HSM); Tyrosine decarboxylase (TDC)

Table 3. Outcomes found in the included studies

Outcomes	Nicotine	Cocaine	Marijuana
% survival	+	+	+
Mortality%	+	-	+
Pupariation%	+	-	+
ET50 (number of days required for 50% of flies to hatch)	+	-	-
Malformation	+	+	-
Weight (mg)	+	-	+
Locomotor Activity	+	+	+
Cerebral hemisphere area (mm2)	+	-	-
Number of positive neurons for tyrosine hydroxylase	+	+	-
Time to 50% sedation (ST50)	+	-	-
Climbing agility%	+	+	+
Ability to fly (%)	+	-	-
Enzyme activity (catalase, SOD, Glutathione - (unit / mg protein))	+	-	+
Behavior alterations	-	+	-
Body fat content (ug / ug / fly)	+	+	+
Differential gene expression	+	+	+
% of impaired Drosophilas	-	-	-
Cardiac function (transient calcium)	+	-	+
Reactive Oxygen species (DCF umol)	-	-	-
Oxidative stress	+	-	+
Number of eclosed flies	+	-	+

Morphological changes of flies after exposure to drugs of abuse

Studies with flies exposed to nicotine have shown anatomical malformation (n = 2), as well as changes in weight (n = 2), cerebral hemisphere area (n = 1), and body fat content (n = 1). In contrast, articles with exposure to cocaine showed only malformation, ovarian defects (n = 1) and changes in body fat content (n = 2). Finally, Marijuana studies have shown weight (n = 1) and body fat content (n = 2).

Molecular and physiological changes in flies exposed to drugs of abuse

Some articles approached molecular changes in flies followed by exposure to nicotine, including alterations in the number of positive neurons for tyrosine hydroxylase (n = 2), enzyme activity (Superoxide dismutase activity and Levels of total glutathione) (n = 1), differential gene expression (Nrf2, Number of transcripts correlated with survival time or larval resistance to nicotine, CG14691, Ugt86Dd, CYP6G1, Cyp18a1 expression, Upregulation of Hsp70, GSH

metabolism (gstD4, gstD5, gstD8), the CYP metabolism (cyp6a2, cyp18a1, cyp307a2), and the oxidative stress response (hsp27, hsp40, hsp70)) (n = 9), and oxidative stress (Level of 8-hydroxydeoxyguanosine, Purine base levels (Uric acid, Xanthine, Hypoxanthine) (n = 2). One study using flies exposed to cocaine observed differential gene expression (Cluster specific coexpression affected by acute cocaine exposure) and another the number of positive neurons for tyrosine hydroxylase. One study using flies exposed to marijuana showed changes in cardiac function (SERCA activity and heart rate), oxidative stress (malondialdehyde) and enzyme activity (SOD, CAT, GSH).

Flies behavior after exposure to drugs of abuse

Changes in climbing agility have been observed in studies with D. melanogaster exposed to nicotine (n = 6), cocaine (n = 6), and marijuana (n = 1). On the other hand, articles showed that the variable time to 50% sedation (ST50) (n = 1) and ability to fly (n = 2) was only interfered by the exposure of flies to nicotine.

Development of flies exposed to drugs of abuse

Studies have shown that exposure of flies to nicotine led to mortality (n = 1), as well as changes in pupariation (n = 1) and number of eclosed flies (n = 2). In addition, articles with flies exposed to marijuana showed changes in the mortality rate (n = 2), number of eclosed flies (n = 1) and pupariation (n = 2).

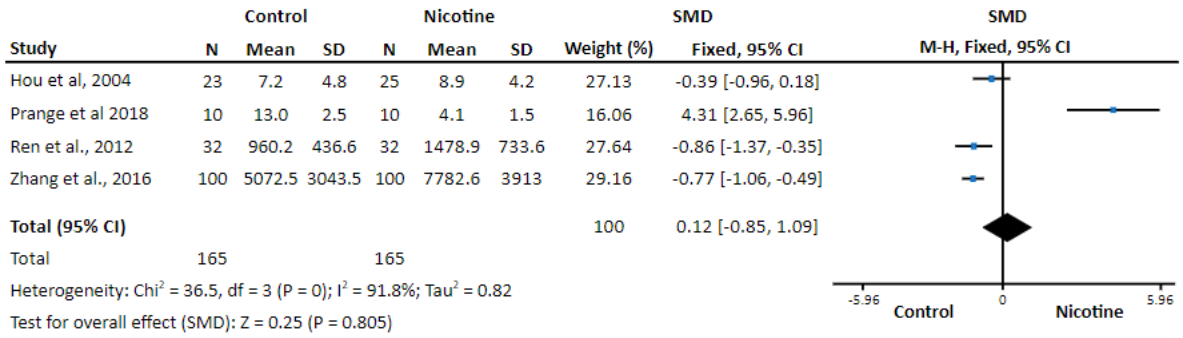
3.2 Inclusion in meta-analysis

Locomotor activity of flies exposed to drugs of abuse

Changes in locomotor activity were described in 12 papers, which investigated exposures to nicotine (n = 5), cocaine (n = 7) and marijuana (n=1). Of which, 10 studies provided appropriate data to be included in the meta-analysis, 4 on nicotine and 6 on cocaine.

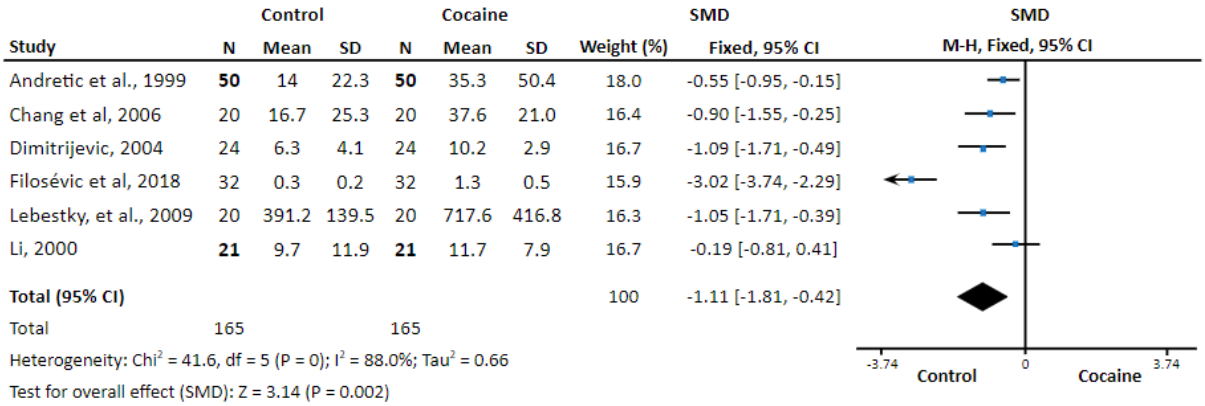
Three studies showed an increase in locomotor activity while one study showed a decrease in this parameter after flies' exposure to nicotine. In the meta-analysis this contributed to a non-significant result (SMD: 0.123 [95% CI: -0.853; 1.099]; $I^2 = 91.8\%$) (Fig. 2).

Figure 2. Locomotor activity exposure of flies to nicotine



In a sensitivity analysis, excluding the study by Prange et al. (2018), the heterogeneity of these studies was reversed and there was statistical significance between the groups (SMD: -0.72 [95% CI: -0.95; - 0.59]; $P < 0.01$; $I^2 = 0\%$; $P = 0.42$). Similarly, the other 6 articles reported a statistically significant hyperactivity in D. melanogaster exposed to cocaine (SMD = -1.11, [95% CI: -1.81, -0.42], P = 0.002, I2 = 88%) (Fig. 3).

Figure 3 - Locomotor activity exposure of flies to Cocaine

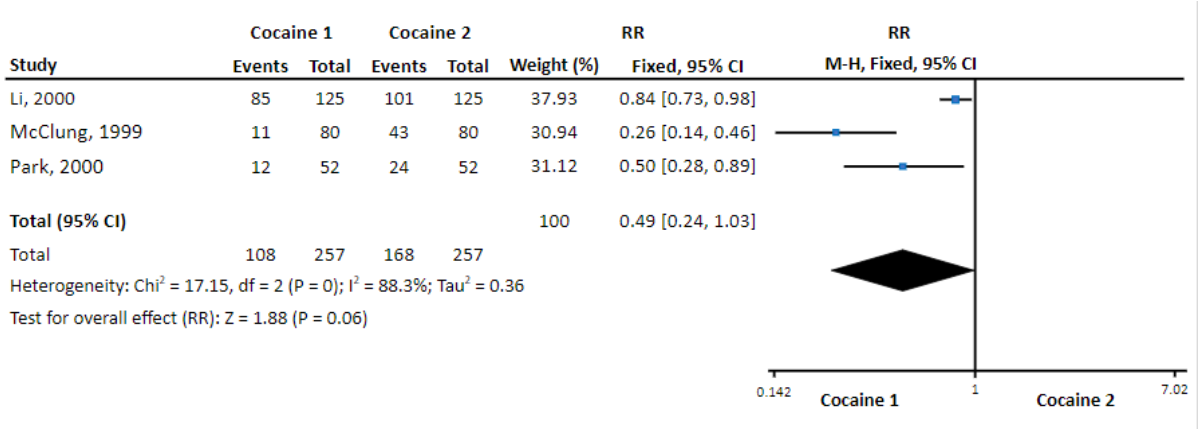


Behavior alterations of flies after exposure to drugs of abuse

Behavioral changes in flies were shown in a total of 7 articles with exposure to cocaine. Of which, only 3 were suitable for meta-analysis. These 3 papers reported no statistically

significant behavioral changes in flies followed by cocaine exposure (RR = 0.5, [95% CI: 0.24, 1.03], P = 0.06, I2 = 88,3 %, P = 0.00) (Fig. 4).

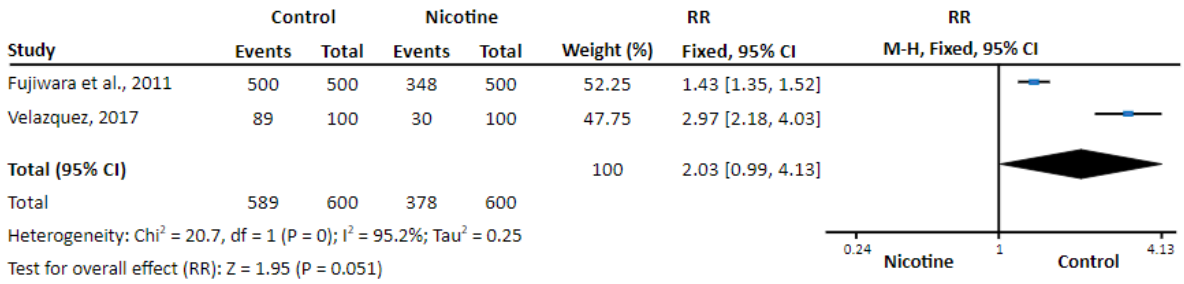
Figure 4 - Behavioral changes in flies with exposure to cocaine



Survival of flies after exposure to drugs of abuse

The percentage of fly survival was observed in a total of 14 articles that evaluated exposure to nicotine (n = 13), cocaine (n = 1), and marijuana (n = 2). Out of that, only 2 studies on nicotine exposure, presenting 2 comparison groups (exposed and not exposed), were included in the meta-analysis. These 2 articles showed a decrease in the percentage of survival (not statistically significant) following the exposure of D. melanogaster to nicotine (RR = 2.03, [95% CI: 0.99, 4.13], P = 0.051, I2 = 95.2%) (Fig. 5).

Figure 5 - Survival of flies after exposure to nicotine



ET50 of flies after exposure to drugs of abuse

Finally, two articles included that addressed the ET50 variable met the criteria for meta-analysis. These studies showed an increase in time to 50% eclosion (not statistically significant) after exposure of the flies to nicotine (SMD = -3.35, [95% CI: -7.77, 1.08], P = 0.14, I2 = 94.4 %) (Fig. 6).

Figure 6 - ET50 of flies after exposure to nicotine

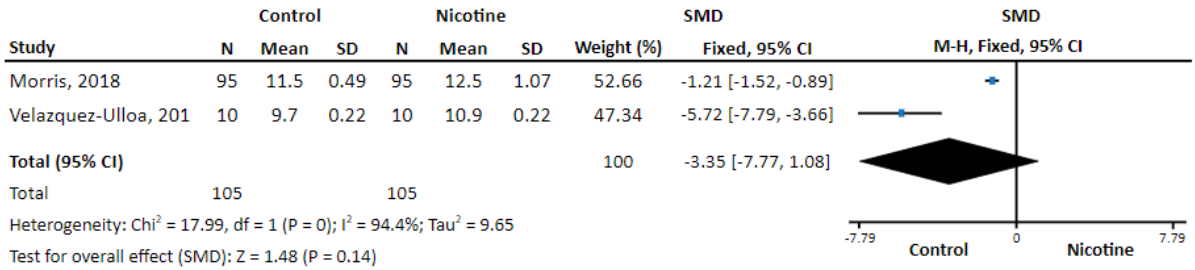


Table 4. Analysis of the study quality according to the modified CAMARADES tool

Author	Year	1	2	3	4	5	6	7	8	9	10	Quality score
Cocaine exposure in Drosophila melanogaster												
Mcclung and Hirsh	1998	Y	N	Y	Y	Y	Y	Y	N	Y	N	7
Torres and Horowitz	1998	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Mcclung and Hirsh	1999	Y	Y	Y	Y	Y	Y	Y	N	Y	N	8
Andretic et al.	1999	Y	N	Y	N	Y	Y	Y	Y	Y	N	7
Bainton et al.	2000	Y	Y	Y	N	Y	Y	Y	Y	Y	N	8
Park et. al.	2000	Y	Y	Y	N	Y	Y	Y	N	Y	N	7
Li et al.	2000	Y	Y	Y	Y	Y	Y	Y	N	Y	N	8
Dimitrijevic et al.	2004	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Tsai et al.	2004	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Bainton et al.	2005	Y	Y	Y	Y	Y	Y	Y	N	Y	N	8
Lease and Hirsh	2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Chang et al.	2006	Y	N	Y	Y	Y	Y	Y	Y	Y	N	8
Sedore willard et al.	2006	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Hardie et al.	2007	Y	Y	Y	Y	Y	Y	Y	N	Y	N	8
Lebestky et al.	2009	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Gakamsky et al.	2013	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Filoševic et al.	2018	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Philipsen et. al.	2018	Y	N	Y	Y	Y	Y	Y	Y	Y	N	8
Philipsen et. al.	2020	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Baker et. al.	2021	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Nicotine exposure in Drosophila melanogaster												
Carrillo and Gibson	2002	Y	Y	N	Y	Y	Y	Y	N	Y	N	7
Hou et. al.	2004	Y	N	Y	N	Y	Y	Y	Y	Y	N	7
Passador-Gurgel et. al.	2007	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	9
Gui and Grant	2008	Y	Y	N	Y	Y	Y	Y	Y	Y	N	8
Hamatake et al.	2009	Y	Y	Y	Y	Y	Y	N	N	Y	N	7
Fujiwara et al.	2011	Y	Y	Y	Y	-	Y	N	Y	Y	N	7
Li et. al.	2012	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Ren et al.	2012	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Chambers et. al.	2013	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Fuenzalida-Uribe et. al.	2013	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	9
Hill-Burns et. al.	2013	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Sadiq and Altaany	2013	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Marriage et. al	2014	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Sanchez-Díaz et. al.	2015	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Uchiyama et al.	2015	Y	Y	Y	Y	Y	Y	N	N	N	Y	7
Zhang et. al.	2016	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	9
Highfill et. al.	2016	Y	Y	Y	Y	-	Y	Y	Y	Y	N	8
Velazquez-Ulloa	2017	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	9
Prange et. al.	2018	Y	N	Y	Y	Y	Y	N	Y	Y	Y	8
Morris et. al.	2018	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Macdonald and Highfill	2020	Y	N	Y	Y	-	Y	Y	Y	Y	Y	8
Santalla et. al.	2021	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	9

Carvajal-Oliveros et. al.	2021	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
El-Merhie et. al.	2021	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Mannett et. al.	2022	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Sirocko et. al.	2022	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Marijuana exposure in Drosophila melanogaster												
Lee et. al.	2010	Y	Y	Y	Y	Y	Y	N	Y	Y	N	8
Lee at. al.	2011	Y	N	Y	Y	Y	Y	N	Y	Y	N	7
Gómez et. al.	2019	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Yejin Ahn et. al.	2021	Y	Y	Y	Y	Y	Y	Y	y	y	y	10
Vitorovi´c et. al.	2021	Y	Y	Y	Y	-	Y	Y	Y	Y	Y	9

1) Peer reviewed publication;

2) Control of temperature;

3) Description of the strain in the control and exposed groups;

4) Description of age in the control and exposed groups;

5) Description of and sex (only for adult) in the control and exposed groups;

6) Frequency and duration of exposure to drugs of abuse;

7) Dose of abuse drugs for exposure;

8) Control group (not exposed to drugs of abuse) for comparison;

9) Sample number;

10) Statement of potential conflict of interests.

4 DISCUSSION

For the first time, a systematic review and meta-analysis showed the effects of psychoactive drugs and their underlying mechanisms on the development and behavior of D. melanogaster. The reduced number of articles on marijuana, tobacco and cocaine, the heterogeneity of the studies and the insufficient number of articles describing the variables included made it difficult to perform a meta-analysis for all variables.

Locomotor activity was increased after nicotine exposure in D. melanogaster (not statistically significant) (Hou et al. 2004; Ren et al. 2012b; Zhang et al. 2016). However, one study used chronic exposure and a high dose of nicotine, indicating the heterogeneity of the articles (Prange et al. 2018). By excluding this study to analyze sensitivity, it was possible to reverse the heterogeneity, indicating that flies show locomotor hyperactivity when exposed to nicotine ($P < 0.05$; Fig. 2). The heterogeneity of the routes of administration, nicotine concentration and the exposure time was a limiting factor in these studies. Similarly, cocaine exposure also increased locomotor activity in D. melanogaster (Andretic 1999; Li et al. 2000; Dimitrijevic et al. 2004; Chang et al. 2006; Lebestky et al. 2009; Filošević et al. 2018). Although we observed a significant result in the meta-analysis ($P < 0.05$; Fig. 3), the high heterogeneity of the studies is undeniable, due to the use of different routes of administration, cocaine concentration and strains. Concentration-dependent exposure to cocaine caused a transient increase in locomotor activity, representing an acute dose sensitivity. Interestingly, the increase in locomotion occurred at the lowest doses tested, intensifying with a second exposure (Filošević et al. 2018). Therefore, our analysis indicated that exposure to nicotine and cocaine is capable of potentiating locomotor behavior.

The increase in locomotor activity observed in flies exposed to cocaine may be influenced by mutations in the expression of several genes (Filošević et al. 2018). OregonR flies mutated for the per gene showed no locomotor changes when exposed to cocaine (75 µg), unlike non-mutant flies (Andretic 1999). In view of this, biological clock genes (such as per) seem to be involved in cocaine sensitization in Drosophila. In contrast, another study observed that cocaine injection (200 pmol per fly) promoted a dose-dependent increase in locomotor activity in period null (per0) mutant flies, similarly to wild-type Canton S flies, without producing sensitization when submitted to repeated injections of cocaine (Dimitrijevic et al. 2004) . Furthermore, the LIM-only (LMO) gene is able to encode a protein regulator of the LIM homeodomain and its mutation can alter cocaine sensitivity in Drosophila. When LMO function is decreased, an increase in the behavioral responses to cocaine is observed, however the overexpression of this gene reduces the responsiveness to cocaine (Tsai et al. 2004). Therefore,

circadian rhythms may be involved in the control of cocaine responsiveness and sensitivity.

A change in locomotor parameters after cocaine exposure was also observed by the negative geotaxis test. Drosophila Canton-S treated with cocaine (100 to 500 μg) shows abnormal locomotion in the negative geotaxis test, with a dose-dependent reduction in this response compared to control flies (Bainton et al. 2000). Reduced climbing ability due to volatilized cocaine was also seen in Male flies carrying the X-linked EP1529 insertion (Drosophila mutants with more rapid responses to volatilized free base cocaine), demonstrating cocaine sensitization. This indicates that this drug is capable of generating significant behavioral changes, especially regarding the locomotion and sensitization of Drosophila (Bainton et al. 2005).

The motor-activating effects of cocaine are sexually dimorphic and require a functional dopaminergic transporter (Filošević et al. 2018). One study showed that male Canton S flies exposed to agude cocaine (0.53 μL) had a slowing in the climbing response, a fact not verified in females. In addition, males and females exposed to cocaine showed a lower rate of locomotion when compared to controls, which can be attributed to an increase in the frequency of grooming, especially in exposed males (Baker et al. 2021). Similarly, using male Drosophila from different strains (Oregon R (WT); Ddc-GAL4; UAS-DVMAT), another study observed that exposure to volatilized cocaine (200 mg) also reduced the climbing response of flies when compared to WT. These negative effects on the climbing response were also observed in Lmo mutant flies (Tsai et al. 2004).

Some studies have evaluated behavioral sensitization, a behavior that is defined as an increased motor-stimulant response due to repeated and intermittent exposure to most drugs of abuse, such as cocaine. For this, flies were exposed to 2 doses of cocaine separated by an interval of 6 h (McClung and Hirsh 1999; Li et al. 2000; Park et al. 2000). Regarding the second exposure to cocaine, no significant change was observed in our analysis ($P > 0.05$; Fig. 4) due to the heterogeneity of the studies. However, when looking at the studies in isolation, there is an increased response of behavioral changes (rapid twirling, erratic jumping, or paralysis) related to the second exposure to cocaine. An interesting study showed that inactive mutant (iav) male flies, which have a low level of octapamine in the central nervous system, failed to sensitize to cocaine in contrast to wild-type flies (McClung and Hirsh 1999). However, the response to initial cocaine exposure was maintained in both strains. Based on these results, the authors suggested that octopamine is not required for sensitization (McClung and Hirsh 1999).

Some signaling pathways have been associated with sensitization to cocaine exposure in Drosophila. PKA signaling pathway appears to play a pivotal role in modulating responses

to chronic cocaine administration in Drosophila flies. Changes in this pathway have already been related to lower sensitization and locomotor activity, requiring higher doses to induce behavioral change without promoting sensitization (Park et al. 2000).

The aminergic signaling pathways are also the target of research that seeks to establish their relationship with cocaine use. Increased aminergic signaling is known to exacerbate locomotion in flies, presumably due to a higher concentration of extracellular amine (Yamamoto and Seto 2014). Furthermore, the number of monoamine neurotransmitters, including dopamine (DA) and serotonin (5HT), can be modulated in mammals by vesicular monoamine transporters (VMATs) (Aggarwal and Mortensen 2017). It is known that a number of Drosophila behaviors can be regulated by DA and 5HT (Vallés and White, 1988; Yellman et al., 1997). Interestingly, one study showed that Drosophila isoform of VMAT (DVMAT) overexpression, expressed in dopaminergic and serotonergic neurons, can increase locomotor activity and grooming behavior (Chang et al. 2006). These authors observed that the use of inhibitors of DVMAT activity and DA receptors can reverse these behaviors. Similarly, another study fed cocaine to DA receptor mutant flies (DopR) and also observed the centrality of biogenic amines in the effects of cocaine on Drosophila (Lebestky et al. 2009). Furthermore, cell signaling modulated in dopaminergic and serotonergic neurons were also related to sensitization behavior (Li et al. 2000). The expression of a stimulatory (s) or inhibitory (i) G alpha subunit, or of the tetanus toxin (TNT) light chain in dopaminergic and serotonergic neurons of flies was able to block the sensitization behavior to repeated exposure to cocaine. However, these authors found that repeated exposures to cocaine were not sufficient to generate sensitization. In other words, understanding the signaling pathways underlying drug abuse exposure is crucial to supporting behavioral changes.

When we analyzed the effect of nicotine exposure on survival parameters ($P > 0.05$; Fig. 5) and time to 50% eclosion (ET50; $P > 0.05$; Fig. 6), no statistical difference was observed in the meta-analysis. This result can be supported by the heterogeneity of the studies, since there are different types of strains, exposure time and administration routes. Typically, high mortality, developmental delay and reduced birth weight have been observed in humans and rodents due to nicotine exposure (Cornelius and Day 2000) Rehman et al., 2021; Rupprecht et al., 2018; Siqueira, 2017). Using Drosophila larvae (3rd stage) exposed to environmental cigarette smoke for 2, 4, or 6 h, one study showed that larval survival to adulthood was reduced as exposure time increased (Fujiwara et al. 2011). According to these authors, the metamorphosis from larva to pupa was inhibited, making it difficult to reach the adult stage. Although the type, dose, route and frequency of exposure were different, other studies reported similar outcomes (Velazquez-

Ulloa 2017; Morris et al. 2018). The Velasquez-Ulloa study exposed Drosophila flies throughout development, from egg to 2 days after eclosion, to a food with an increasing concentration of nicotine (0.1 – 0.4 mg/ml). This researcher observed that higher concentrations of nicotine (0.3 and 0.4 mg/ml) gradually reduced the survival rate by 16 days after egg-laying, because of the lethality in the larval, not the pupal, stages (Velazquez-Ulloa 2017). In addition, a developmental delay (ET50 > 10.5 days) proportional to increasing nicotine concentrations was also observed in this study. Similarly, in another study of this group, using the same dose, route and exposure time, the authors observed the same changes (Morris et al. 2018). Although our analyzes regarding survival and ET50 were not significant, nicotine exposure promotes changes in these parameters when the studies are evaluated individually.

Studies with exposure to marijuana did not present a profile for meta-analysis due to the low rate found in the databases that met the pre-established inclusion criteria (Lee et al. 2010; Lee et al. 2011; Gómez et al. 2019). However, exposure of Drosophila flies to marijuana promotes changes in parameters not discussed above. Marijuana exposure can lead to some cardiovascular changes. Studies in patients show that exposure to smoked marijuana induces heart rhythm abnormalities (Johnson and Domino 1971; AKINS and AWDEH 1981; Singh 2000; Kosior et al. 2001; Fisher 2005; Rezkalla and Kloner 2019). In a study with Drosophila, exposure to marijuana vapor (two daily doses of 15 minutes for 5, 6–8 or 11–13 days) potentiated the arrhythmicity index (6-8 days) early and transiently, an effect not observed at long term (11-13 days) (Gómez et al. 2019). Based on these data, short-term exposure appears to be deleterious because of the arrhythmic patterns of cardiac activity associated with increased fly mortality. Possibly, these heart arrhythmias are an initial response as an adaptive mechanism in order to reach a steady state after cannabis consumption (Gómez et al. 2019). In addition, these authors observed a high contractility in the longer period of exposure to marijuana, without changing the heart rate. Corroborating this data, they found an increase in the amplitude of the calcium transient, but without altering the accumulation of calcium in the sarcoplasmic reticulum, the fractional release, the activity of the sarcoplasmic reticulum calcium ATPase pumpe and sodium/calcium exchanger in the Drosophila heart.

Studies with hempseed were also included in our analysis due to the low number of papers found that matched the eligible inclusion criteria. Hempseed (Cannabis sativa L.) has shown benefits for nutrition and health, with a pharmaceutical potential, which has increased the interest in its consumption by the population in several countries (Leonard et al. 2020; Cerino et al. 2021). In contrast to marijuana, hempseed contains a very small amount of tetrahydrocannabinol (THC), which promotes the psychotropic effects of Cannabis (Jang et al.

2020). A study with hempseed meal (HSM) intake in Drosophila showed an increase in the body weight of flies, possibly due to the presence of cholesterol in the hempseed (Lee et al. 2010). Furthermore, these authors observed that HSM increased wing length, reduced pupation and egg laying time, but survival and longevity remained unchanged. Finally, this same research group showed in another study that HSM can be a strong alternative to treat cardiovascular problems and neurodegenerative diseases such as Alzheimer's and Parkinson's (Lee et al. 2011).

CONCLUSION

Our results demonstrate that exposure to psychoactive drugs such as cocaine, nicotine and marijuana causes morphological, developmental, behavioral and molecular changes. In the meta-analysis, we observed that flies exposed to cocaine have greater locomotor activity. Furthermore, from this review, it is evident that D. Melanogaster is a useful model for understanding the mechanisms underlying the use of drugs of abuse. However, important questions about drug use remain unanswered in flies. It would be interesting to use existing assays to further study and then develop new and more complex behavioral assays using females and offspring to answer new questions. We suggest that researchers better describe the methodological design in their work, temperature, the strain used, sex, lifespan, etc. This will facilitate the understanding of the work, the development of future reviews with meta-analysis and the replication of methodologies.

However, our report can help researchers in the area design future experiments.

Author Contributions

OWC, LAA, JFS, IRRB and ISM: data collection, project development, manuscript writing and editing, data administration, data interpretation.

NBB: project development, data administration, data interpretation, data analysis, statistical analysis.

OWC, JFS, ISM, KBO and LAA: project development, scientific knowledge, data collection, project development, manuscript writing and editing.

Disclosure of interest

The authors declare that they have no conflict of interest.

References

Aggarwal S, Mortensen O v. 2017. Overview of Monoamine Transporters. Curr Protoc Pharmacol. 79:12.16.1-12.16.17. https://doi.org/10.1002/cpph.32

AKINS D, AWDEH MR. 1981. Marijuana and Second-Degree AV Block. South Med J [Internet]. 74(3):371–372. https://doi.org/10.1097/00007611-198103000-00035

Andretic R. 1999. Requirement of Circadian Genes for Cocaine Sensitization in Drosophila. Science (1979) [Internet]. 285(5430):1066–1068. https://doi.org/10.1126/science.285.5430.1066

Ankit Rohatgi. 2022. WebPlotDigitizer [Internet]. [accessed 2022 Mar 2]. https://automeris.io/WebPlotDigitizer/

Ayala M. 2009. Brain Serotonin, Psychoactive Drugs, and Effects on Reproduction. Cent Nerv Syst Agents Med Chem [Internet]. 9(4):258–276. https://doi.org/10.2174/187152409789630389

Bainton RJ, Tsai LT, Singh CM, Moore MS, Neckameyer WS, Heberlein U. 2000. Dopamine modulates acute responses to cocaine, nicotine and ethanol in Drosophila. Curr Biol [Internet]. 10(4):187–94. https://doi.org/10.1016/s0960-9822(00)00336-5

Bainton RJ, Tsai LT-Y, Schwabe T, DeSalvo M, Gaul U, Heberlein U. 2005. moody Encodes Two GPCRs that Regulate Cocaine Behaviors and Blood-Brain Barrier Permeability in Drosophila. Cell [Internet]. 123(1):145–156. https://doi.org/10.1016/j.cell.2005.07.029

Baker BM, Mokashi SS, Shankar V, Hatfield JS, Hannah RC, Mackay TFC, Anholt RRH. 2021. The Drosophila brain on cocaine at single-cell resolution. Genome Res. 31(10):1927–1937. https://doi.org/10.1101/gr.268037.120

Cerino P, Buonerba C, Cannazza G, D'Auria J, Ottoni E, Fulgione A, di Stasio A, Pierri B, Gallo A. 2021. A Review of Hemp as Food and Nutritional Supplement. Cannabis Cannabinoid Res. 6(1):19–27. https://doi.org/10.1089/can.2020.0001

Chang H-Y, Grygoruk A, Brooks ES, Ackerson LC, Maidment NT, Bainton RJ, Krantz DE. 2006. Overexpression of the Drosophila vesicular monoamine transporter increases motor activity and courtship but decreases the behavioral response to cocaine. Mol Psychiatry. 11(1):99–113. https://doi.org/10.1038/sj.mp.4001742

Chang TR, Kowalski RG, Carhuapoma JR, Tamargo RJ, Naval NS. 2016. Cocaine use as an independent predictor of seizures after aneurysmal subarachnoid hemorrhage. J Neurosurg [Internet]. 124(3):730–735. https://doi.org/10.3171/2015.2.JNS142856

Chen AS, Read RD. 2019. Drosophila melanogaster as a Model System for Human Glioblastomas. Adv Exp Med Biol [Internet]. 1167:207–224. https://doi.org/10.1007/978-3-030-23629-8_12

Chen C-Y, Lin K-M. 2009. Health consequences of illegal drug use. Curr Opin Psychiatry [Internet]. 22(3):287–292. https://doi.org/10.1097/YCO.0b013e32832a2349

Cornelius MD, Day NL. 2000. The effects of tobacco use during and after pregnancy on exposed children. Alcohol Res Health [Internet]. 24(4):242–9. http://www.ncbi.nlm.nih.gov/pubmed/15986719

Crivelaro do Nascimento G, Ferrari DP, Guimaraes FS, del Bel EA, Bortolanza M, Ferreira-Junior NC. 2020. Cannabidiol increases the nociceptive threshold in a preclinical model of Parkinson's disease. Neuropharmacology [Internet]. 163:107808. https://doi.org/10.1016/j.neuropharm.2019.107808

Crocq M-A. 2007. Historical and cultural aspects of man's relationship with addictive drugs. Dialogues Clin Neurosci [Internet]. 9(4):355–61. http://www.ncbi.nlm.nih.gov/pubmed/18286796

Dimitrijevic N, Dzitoyeva S, Manev H. 2004. An automated assay of the behavioral effects of cocaine injections in adult Drosophila. J Neurosci Methods [Internet]. 137(2):181–4. https://doi.org/10.1016/j.jneumeth.2004.02.023

Filošević A, Al-samarai S, Andretić Waldowski R. 2018. High Throughput Measurement of Locomotor Sensitization to Volatilized Cocaine in Drosophila melanogaster. Front Mol Neurosci [Internet]. 11. https://doi.org/10.3389/fnmol.2018.00025

Fisher BAC. 2005. Cardiovascular complications induced by cannabis smoking: a case report and review of the literature. Emergency Medicine Journal [Internet]. 22(9):679–680. https://doi.org/10.1136/emj.2004.014969

le Foll B, Goldberg SR. 2009. Effects of nicotine in experimental animals and humans: an update on addictive properties. Handb Exp Pharmacol [Internet].(192):335–67. https://doi.org/10.1007/978-3-540-69248-5_12

Fujiwara M, Hamatake Y, Arimoto S, Okamoto K, Suzuki T, Negishi T. 2011. Exposure to Cigarette Smoke Increases Urate Level and Decreases Glutathione Level in Larval Drosophila melanogaster. Genes and Environment [Internet]. 33(3):89–95. https://doi.org/10.3123/jemsge.33.89

Gatch MB, Dolan SB, Forster MJ. 2016. Locomotor, discriminative stimulus, and place conditioning effects of MDAI in rodents. Behavioural Pharmacology [Internet]. 27(6):497–505. https://doi.org/10.1097/FBP.0000000000000237

Ghezzi A, Atkinson NS. 2011. Homeostatic control of neural activity: a Drosophila model for drug tolerance and dependence. Int Rev Neurobiol [Internet]. 99:23–50. https://doi.org/10.1016/B978-0-12-387003-2.00002-1

Goldstein RA, DesLauriers C, Burda A, Johnson-Arbor K. 2009. Cocaine: history, social implications, and toxicity: a review. Semin Diagn Pathol [Internet]. 26(1):10–7. https://doi.org/10.1053/j.semdp.2008.12.001

Gómez IM, Rodríguez MA, Santalla M, Kassis G, Colman Lerner JE, Aranda JO, Sedán D, Andrinolo D, Valverde CA, Ferrero P. 2019. Inhalation of marijuana affects Drosophila heart function. Biol Open [Internet]. 8(8). https://doi.org/10.1242/bio.044081

Hardie SL, Zhang JX, Hirsh J. 2007. Trace amines differentially regulate adult locomotor activity, cocaine sensitivity, and female fertility inDrosophila melanogaster. Dev Neurobiol

[Internet]. 67(10):1396–1405. https://doi.org/10.1002/dneu.20459

Heberlein U, Tsai LT-Y, Kapfhamer D, Lasek AW. 2009. Drosophila, a genetic model system to study cocaine-related behaviors: A review with focus on LIM-only proteins. Neuropharmacology [Internet]. 56:97–106. https://doi.org/10.1016/j.neuropharm.2008.07.023

Holbrook BD. 2016. The effects of nicotine on human fetal development. Birth Defects Res C Embryo Today [Internet]. 108(2):181–192. https://doi.org/10.1002/bdrc.21128

Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. 2014. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 14:43. https://doi.org/10.1186/1471-2288-14-43

Hou J, Kuromi H, Fukasawa Y, Ueno K, Sakai T, Kidokoro Y. 2004. Repetitive exposures to nicotine induce a hyper-responsiveness via the cAMP/PKA/CREB signal pathway in Drosophila. J Neurobiol [Internet]. 60(2):249–61. https://doi.org/10.1002/neu.20021

Jang E, Kim H, Jang S, Lee J, Baeck S, In S, Kim E, Kim Y-U, Han E. 2020. Concentrations of THC, CBD, and CBN in commercial hemp seeds and hempseed oil sold in Korea. Forensic Sci Int. 306:110064. https://doi.org/10.1016/j.forsciint.2019.110064

Johnson S, Domino EF. 1971. Some cardiovascular effects of marihuana smoking in normal volunteers. Clin Pharmacol Ther [Internet]. 12(5):762–768. https://doi.org/10.1002/cpt1971125762

Kampman KM. 2019. The treatment of cocaine use disorder. Sci Adv [Internet]. 5(10):eaax1532. https://doi.org/10.1126/sciadv.aax1532

Kaun KR, Devineni A v., Heberlein U. 2012. Drosophila melanogaster as a model to study drug addiction. Hum Genet [Internet]. 131(6):959–975. https://doi.org/10.1007/s00439-012-1146-6

Koppel BS, Samkoff L, Daras M. 1996. Relation of cocaine use to seizures and epilepsy. Epilepsia [Internet]. 37(9):875–8. https://doi.org/10.1111/j.1528-1157.1996.tb00041.x

Kosiba JD, Maisto SA, Ditre JW. 2019. Patient-reported use of medical cannabis for pain, anxiety, and depression symptoms: Systematic review and meta-analysis. Soc Sci Med. 233:181–192. https://doi.org/10.1016/j.socscimed.2019.06.005

Kosior DA, Filipiak KJ, Stolarz P, Opolski G. 2001. Paroxysmal atrial fibrillation following marijuana intoxication: a two-case report of possible association. Int J Cardiol [Internet]. 78(2):183–184. https://doi.org/10.1016/S0167-5273(00)00459-9

Laviola G, Adriani W, Terranova ML, Gerra G. 1999. Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. Neurosci Biobehav Rev [Internet]. 23(7):993–1010. https://doi.org/10.1016/s0149-7634(99)00032-9

Lebestky T, Chang J-SC, Dankert H, Zelnik L, Kim Y-C, Han K-A, Wolf FW, Perona P, Anderson DJ. 2009. Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. Neuron [Internet]. 64(4):522–36. https://doi.org/10.1016/j.neuron.2009.09.031

Lee MJ, Park MS, Hwang S, Hong YK, Choi G, Suh YS, Han SY, Kim Darae, Jeun J, Oh C-T,

et al. 2010. Dietary hempseed meal intake increases body growth and shortens the larval stage via the upregulation of cell growth and sterol levels in Drosophila melanogaster. Mol Cells [Internet]. 30(1):29–36. https://doi.org/10.1007/s10059-010-0085-0

Lee MJ, Park SH, Han JH, Hong YK, Hwang S, Lee S, Kim D, Han SY, Kim ES, Cho KS. 2011. The effects of hempseed meal intake and linoleic acid on Drosophila models of neurodegenerative diseases and hypercholesterolemia. Mol Cells [Internet]. 31(4):337–342. https://doi.org/10.1007/s10059-011-0042-6

Leonard W, Zhang P, Ying D, Fang Z. 2020. Hempseed in food industry: Nutritional value, health benefits, and industrial applications. Compr Rev Food Sci Food Saf. 19(1):282–308. https://doi.org/10.1111/1541-4337.12517

Levine SR, Brust JCM, Futrell N, Ho K-L, Blake D, Millikan CH, Brass LM, Fayad P, Schultz LR, Selwa JF, Welch KMA. 1990. Cerebrovascular Complications of the Use of the Crack Form of Alkaloidal Cocaine. New England Journal of Medicine [Internet]. 323(11):699–704. https://doi.org/10.1056/NEJM199009133231102

Li H, Chaney S, Forte M, Hirsh J. 2000. Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. Current Biology [Internet]. 10(4):211–214. https://doi.org/10.1016/S0960-9822(00)00340-7

Macleod MR, O'Collins T, Howells DW, Donnan GA. 2004. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke [Internet]. 35(5):1203–8. https://doi.org/10.1161/01.STR.0000125719.25853.20

Maia RM, Valente V, Cunha MA, Sousa JF, Araujo DD, Silva WA, Zago MA, Dias-Neto E, Souza SJ, Simpson AJ, et al. 2007. Identification of unannotated exons of low abundance transcripts in Drosophila melanogaster and cloning of a new serine protease gene upregulated upon injury. BMC Genomics [Internet]. 8(1):249. https://doi.org/10.1186/1471-2164-8-249

Matsuo R, Yamagishi M, Wakiya K, Tanaka Y, Ito E. 2013. Target innervation is necessary for neuronal polyploidization in the terrestrial slug Limax. Dev Neurobiol [Internet]. 73(8):609–20. https://doi.org/10.1002/dneu.22087

McClung C, Hirsh J. 1999. The trace amine tyramine is essential for sensitization to cocaine in Drosophila. Curr Biol [Internet]. 9(16):853–60. https://doi.org/10.1016/s0960-9822(99)80389-3

McPartland JM, Matias I, di Marzo V, Glass M. 2006. Evolutionary origins of the endocannabinoid system. Gene [Internet]. 370:64–74. https://doi.org/10.1016/j.gene.2005.11.004

Merigian KS, Park LJ, Leeper K v, Browning RG, Giometi R. Adrenergic crisis from crack cocaine ingestion: report of five cases. J Emerg Med [Internet]. 12(4):485–90. https://doi.org/10.1016/0736-4679(94)90345-x

Morris M, Shaw A, Lambert M, Perry HH, Lowenstein E, Valenzuela D, Velazquez-Ulloa NA. 2018. Developmental nicotine exposure affects larval brain size and the adult dopaminergic system of Drosophila melanogaster. BMC Dev Biol [Internet]. 18(1):13. https://doi.org/10.1186/s12861-018-0172-6

Nahas G, Latour C. 1992. The human toxicity of marijuana. Med J Aust [Internet]. 156(7):495–7. http://www.ncbi.nlm.nih.gov/pubmed/1313532

Neiman J, Haapaniemi HM, Hillbom M. 2000. Neurological complications of drug abuse: pathophysiological mechanisms. Eur J Neurol [Internet]. 7(6):595–606. https://doi.org/10.1046/j.1468-1331.2000.00045.x

de Nobrega AK, Lyons LC. 2017. Drosophila: An Emergent Model for Delineating Interactions between the Circadian Clock and Drugs of Abuse. Neural Plast [Internet]. 2017:4723836. https://doi.org/10.1155/2017/4723836

Pacheco ALD, de Melo IS, de Souza FMA, Nicácio DCSP, Freitas-Santos J, Oliveira Dos Santos YM, Costa M de A, Cavalcante C de MB, Gomes Dos Santos Neto J, Gitaí DLG, et al. 2021. Maternal crack cocaine use in rats leads to depressive- and anxiety-like behavior, memory impairment, and increased seizure susceptibility in the offspring. Eur Neuropsychopharmacol [Internet]. 44:34–50. https://doi.org/10.1016/j.euroneuro.2020.12.011

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, et al. 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ.:n71. https://doi.org/10.1136/bmj.n71

Pandey UB, Nichols CD. 2011. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev [Internet]. 63(2):411–36. https://doi.org/10.1124/pr.110.003293

Park SK, Sedore SA, Cronmiller C, Hirsh J. 2000. Type II cAMP-dependent protein kinase-deficient Drosophila are viable but show developmental, circadian, and drug response phenotypes. J Biol Chem [Internet]. 275(27):20588–96. https://doi.org/10.1074/jbc.M002460200

Philipsen MH, Phan NTN, Fletcher JS, Malmberg P, Ewing AG. 2018. Mass Spectrometry Imaging Shows Cocaine and Methylphenidate Have Opposite Effects on Major Lipids in Drosophila Brain. ACS Chem Neurosci [Internet]. 9(6):1462–1468. https://doi.org/10.1021/acschemneuro.8b00046

Prange R, Thiedmann M, Bhandari A, Mishra N, Sinha A, Häsler R, Rosenstiel P, Uliczka K, Wagner C, Yildirim AÖ, et al. 2018. A Drosophila model of cigarette smoke induced COPD identifies Nrf2 signaling as an expedient target for intervention. Aging [Internet]. 10(8):2122–2135. https://doi.org/10.18632/aging.101536

Ren J, Sun J, Zhang Y, Liu T, Ren Q, Li Y, Guo A. 2012a. Down-regulation of Decapping Protein 2 mediates chronic nicotine exposure-induced locomotor hyperactivity in Drosophila. PLoS One [Internet]. 7(12):e52521. https://doi.org/10.1371/journal.pone.0052521

Ren J, Sun J, Zhang Y, Liu T, Ren Q, Li Y, Guo A. 2012b. Down-regulation of Decapping Protein 2 mediates chronic nicotine exposure-induced locomotor hyperactivity in Drosophila. PLoS One [Internet]. 7(12):e52521. https://doi.org/10.1371/journal.pone.0052521

Rezkalla S, Kloner RA. 2019. Cardiovascular effects of marijuana. Trends Cardiovasc Med [Internet]. 29(7):403–407. https://doi.org/10.1016/j.tcm.2018.11.004

Rodríguez-Muñoz M, Onetti Y, Cortés-Montero E, Garzón J, Sánchez-Blázquez P. 2018.

Cannabidiol enhances morphine antinociception, diminishes NMDA-mediated seizures and reduces stroke damage via the sigma 1 receptor. Mol Brain [Internet]. 11(1):51. https://doi.org/10.1186/s13041-018-0395-2

Ryan SA. 2019. Cocaine Use in Adolescents and Young Adults. Pediatr Clin North Am [Internet]. 66(6):1135–1147. https://doi.org/10.1016/j.pcl.2019.08.014

dos Santos JF, de Melo Bastos Cavalcante C, Barbosa FT, Gitaí DLG, Duzzioni M, Tilelli CQ, Shetty AK, de Castro OW. 2018. Maternal, fetal and neonatal consequences associated with the use of crack cocaine during the gestational period: a systematic review and meta-analysis. Arch Gynecol Obstet [Internet]. 298(3):487–503. https://doi.org/10.1007/s00404-018-4833-2

Shibuya T, Nakane T, Takano A, Yamauchi J, Morimoto T. 2021. Dopamine modulation of chemotactic behavior in response to natural aroma substances in Drosophila melanogaster larvae. Phytomedicine Plus [Internet]. 1(1):100007. https://doi.org/10.1016/j.phyplu.2020.100007

Singh GK. 2000. Atrial Fibrillation Associated with Marijuana Use. Pediatr Cardiol [Internet]. 21(3):284–284. https://doi.org/10.1007/s002460010063

Sordo L, Indave BI, Degenhardt L, Barrio G, Kaye S, Ruíz-Pérez I, Bravo MJ. 2013. A systematic review of evidence on the association between cocaine use and seizures. Drug Alcohol Depend [Internet]. 133(3):795–804. https://doi.org/10.1016/j.drugalcdep.2013.08.019

Tsai LT-Y, Bainton RJ, Blau J, Heberlein U. 2004. Lmo mutants reveal a novel role for circadian pacemaker neurons in cocaine-induced behaviors. PLoS Biol [Internet]. 2(12):e408. https://doi.org/10.1371/journal.pbio.0020408

Upadhya D, Castro OW, Upadhya R, Shetty AK. 2018. Prospects of Cannabidiol for Easing Status Epilepticus-Induced Epileptogenesis and Related Comorbidities. Mol Neurobiol [Internet]. 55(8):6956–6964. https://doi.org/10.1007/s12035-018-0898-y

Velazquez-Ulloa NA. 2017. A Drosophila model for developmental nicotine exposure.Skoulakis EMC, editor. PLoS One [Internet]. 12(5):e0177710. https://doi.org/10.1371/journal.pone.0177710

Wagenknecht DR, BalHaddad AA, Gregory RL. 2018. Effects of Nicotine on Oral Microorganisms, Human Tissues, and the Interactions between Them. Curr Oral Health Rep [Internet]. 5(1):78–87. https://doi.org/10.1007/s40496-018-0173-3

Wilkinson ST, Yarnell S, Radhakrishnan R, Ball SA, D'Souza DC. 2016. Marijuana Legalization: Impact on Physicians and Public Health. Annu Rev Med [Internet]. 67(1):453–466. https://doi.org/10.1146/annurev-med-050214-013454

Yamamoto S, Seto ES. 2014. Dopamine dynamics and signaling in Drosophila: an overview of genes, drugs and behavioral paradigms. Exp Anim [Internet]. 63(2):107–19. https://doi.org/10.1538/expanim.63.107

Zeng X, Zhang Y, Kwong JSW, Zhang C, Li S, Sun F, Niu Y, Du L. 2015. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. J Evid Based Med [Internet]. 8(1):2–10. https://doi.org/10.1111/jebm.12141

Zhang Y, Guo J, Guo A, Li Y. 2016. Nicotine-induced acute hyperactivity is mediated by dopaminergic system in a sexually dimorphic manner. Neuroscience. 332:149–59. https://doi.org/10.1016/j.neuroscience.2016.06.043

CONCLUSÃO GERAL

O uso abusivo de drogas continua a criar desafios na saúde pública, levando a mortes por overdose e outras condições crônicas de saúde (UNODC, 2022). Dentre as drogas psicoativas, as mais utilizadas são maconha, cocaína e crack (BASTOS, 2017). Nos últimos tempos é observado um padrão de alto consumo de crack em mulheres em idade reprodutiva (HESS; DE ALMEIDA, 2019). A exposição pré-natal ao crack pode ter efeitos significativos no crescimento e no desenvolvimento neurológico do bebê (SINGER; FARKAS; KLIEGMAN, 1992). A ausência de estudos que esclarecessem os mecanismos subjacentes do uso do crack no período gestacional motivou o nosso interesse em investigar na literatura sobre o tema. Nossa primeira revisão sistemática fornece evidências sólidas dos efeitos deletérios do uso de crack na saúde da mãe e do feto, confirmados por meta-análise. Tais efeitos adversos são evidenciados em fetos expostos por pequeno para a idade gestacional, menor peso ao nascer, menor perímetro cefálico, maior ocorrência de descolamento prematuro placentário e parto prematuro(DOS SANTOS et al., 2018). Esforços crescentes devem ser feitos para compreender os resultados do uso de crack, tanto para mães quanto para crianças, para abordar as consequências sociais e de saúde desse comportamento. Os dados encontrados na literatura foram importantes informações para o desenvolvimento de pesquisas pré-clínicas realizadas pelo nosso grupo, as quais deram o ponta pé inicial para preencher as lacunas existentes sobre os mecanismos do crack(PACHECO et al., 2021).

Apesar de o modelo em roedor ser muito bem estabelecido, algumas questões podem ser mais facilmente estudadas em *drosophila melanogaster*. Nossa segunda revisão, a qual deu subsidio para o desenvolvimento de um estudo importante em nosso grupo (dados não publicados), demonstra de forma evidente que *D. Melanogaster* é um modelo útil para entender os mecanismos subjacentes ao uso de drogas de abuso. Nossos resultados demonstram que a exposição a drogas psicoativas como cocaína, nicotina e maconha causa alterações morfológicas, de desenvolvimento, comportamentais e moleculares. Na metanálise, observamos que as moscas expostas à cocaína apresentam maior atividade locomotora (ANDRETIC, 1999; CHANG et al., 2016; DIMITRIJEVIC; DZITOYEVA; MANEV, 2004; FILOŠEVIĆ; AL-SAMARAI; ANDRETIĆ WALDOWSKI, 2018; LEBESTKY et al., 2009; LI et al., 2000). A maioria das variáveis foram discutidas de forma qualitativa, devido ao estudo correspondente não ser elegível para a metánalise. A especificidade de cada estudo promoveu delineamentos experimentais bem diferentes, o que contribuiu para a heterogeneidade entre eles. Porém, fazendo uma análise nos estudos, é observado que doses baixas de nicotina causam aumento da atividade locomotora (HOU et al., 2004; REN et al., 2012; ZHANG et al., 2016) e

doses altas causam diminuição (PRANGE et al., 2018). Apesar de não ser estatisticamente significante, ainda há uma notável diminuição no percentual de sobrevida (FUJIWARA et al., 2011; VELAZQUEZ-ULLOA, 2017) e aumento no tempo para 50% de eclosão após a exposição à nicotina (MORRIS et al., 2018; VELAZQUEZ-ULLOA, 2017). Além disso, também não significativo, há maior mudança de comportamento quando há exposição repetida à cocaína (li 2000, mclung 1999, park 2000).

Nosso trabalho pode ajudar pesquisadores da área a projetar experimentos futuros.

REFERÊNCIAS

ABDALLA, R. R. et al. Prevalence of cocaine use in Brazil: data from the II Brazilian national alcohol and drugs survey (BNADS). **Addictive behaviors**, v. 39, n. 1, p. 297–301, jan. 2014.

ANDRETIC, R. Requirement of Circadian Genes for Cocaine Sensitization in Drosophila. **Science**, v. 285, n. 5430, p. 1066–1068, 13 ago. 1999.

BAINTON, R. J. et al. Dopamine modulates acute responses to cocaine, nicotine and ethanol in Drosophila. **Current biology : CB**, v. 10, n. 4, p. 187–94, 24 fev. 2000.

BAINTON, R. J. et al. moody Encodes Two GPCRs that Regulate Cocaine Behaviors and Blood-Brain Barrier Permeability in Drosophila. **Cell**, v. 123, n. 1, p. 145–156, out. 2005.

BASTOS, F. I. P. M. **III Levantamento Nacional sobre o uso de drogas pela população brasileira**. **FIOCRUZ/ICICT** Rio de Janeiro, 2017.

BELL, G. L.; LAU, K. Perinatal and neonatal issues of substance abuse. **Pediatric clinics of North America**, v. 42, n. 2, p. 261–81, abr. 1990005.

BLAYAC, L. et al. Increase of cannabis and cocaine use by pregnant women in France from 2005 to 2018: Insights of the annual cross sectional OPPIDUM survey. **Therapie**, 8 out. 2022.

BOTELHO, A. P. M.; ROCHA, R. DA C.; MELO, V. H. Uso e dependência de cocaína/crack na gestação, parto e puerpério. **Femina**, v. 41, n. 1, p. 23–32, 2013.

CHANG, T. R. et al. Cocaine use as an independent predictor of seizures after aneurysmal subarachnoid hemorrhage. **Journal of Neurosurgery**, v. 124, n. 3, p. 730–735, mar. 2016.

CRUZ, V. D. et al. Sociodemographic conditions and patterns of crack use among women. **Texto & Contexto - Enfermagem**, v. 23, n. 4, p. 1068–1076, dez. 2014.

DIMITRIJEVIC, N.; DZITOYEVA, S.; MANEV, H. An automated assay of the behavioral effects of cocaine injections in adult Drosophila. **Journal of neuroscience methods**, v. 137, n. 2, p. 181–4, 30 ago. 2004.

DOS SANTOS, J. F. et al. Maternal, fetal and neonatal consequences associated with the use of crack cocaine during the gestational period: a systematic review and meta-analysis. **Archives of gynecology and obstetrics**, v. 298, n. 3, p. 487–503, 2018.

DUAILIBI, L. B.; RIBEIRO, M.; LARANJEIRA, R. Profile of cocaine and crack users in Brazil. **Cadernos de saude publica**, v. 24 Suppl 4, p. s545-57, 2008.

ENGLAND, L. J. et al. Alcohol Use and Co-Use of Other Substances Among Pregnant Females Aged 12-44 Years - United States, 2015-2018. **MMWR. Morbidity and mortality weekly report**, v. 69, n. 31, p. 1009–1014, 2020.

FILOŠEVIĆ, A.; AL-SAMARAI, S.; ANDRETIĆ WALDOWSKI, R. High Throughput Measurement of Locomotor Sensitization to Volatilized Cocaine in Drosophila melanogaster. **Frontiers in Molecular Neuroscience**, v. 11, 5 fev. 2018.

FUJIWARA, M. et al. Exposure to Cigarette Smoke Increases Urate Level and Decreases Glutathione Level in Larval Drosophila melanogaster. **Genes and Environment**, v. 33, n. 3, p. 89–95, 2011.

GANAPATHY, V. Drugs of abuse and human placenta. **Life sciences**, v. 88, n. 21–22, p. 926–30, 23 maio 2011.

GEORGE, O.; KOOB, G. F. Individual differences in the neuropsychopathology of addiction. **Dialogues in Clinical Neuroscience**, v. 19, n. 3, p. 217–229, 30 set. 2017.

GLEA, D. A.; PRESTON, S. H. Estimating the impact of drug use on US mortality, 1999-2016. **PLOS ONE**, v. 15, n. 1, p. e0226732, 15 jan. 2020.

GOMES, E. F. et al. Anhydroecgonine Methyl Ester (AEME), a Product of Cocaine Pyrolysis, Impairs Spatial Working Memory and Induces Striatal Oxidative Stress in Rats. **Neurotoxicity research**, v. 34, n. 4, p. 834–847, nov. 2018.

GÓMEZ, I. M. et al. Inhalation of marijuana affects Drosophila heart function. **Biology open**, v. 8, n. 8, 15 ago. 2019.

HESS, A. R. B.; DE ALMEIDA, R. M. M. Female crack cocaine users under treatment at therapeutic communities in southern Brazil: characteristics, pattern of consumption, and psychiatric comorbidities. **Trends in Psychiatry and Psychotherapy**, v. 41, n. 4, p. 369–374, out. 2019.

HOU, J. et al. Repetitive exposures to nicotine induce a hyper-responsiveness via the cAMP/PKA/CREB signal pathway in Drosophila. **Journal of neurobiology**, v. 60, n. 2, p. 249–61, ago. 2004.

HARDIE, S. L.; ZHANG, J. X.; HIRSH, J. Trace amines differentially regulate adult locomotor activity, cocaine sensitivity, and female fertility inDrosophila melanogaster. **Developmental Neurobiology**, v. 67, n. 10, p. 1396–1405, 1 set. 2007.

HATSUKAMI, D. K.; FISCHMAN, M. W. Crack cocaine and cocaine hydrochloride. Are the differences myth or reality? **JAMA**, v. 276, n. 19, p. 1580–8, 20 nov. 1996.

INCA, N. C. I. **Data and smoking numbers**.

JENNINGS, B. H. Drosophila – a versatile model in biology & medicine. **Materials Today**, v. 14, n. 5, p. 190–195, maio 2011.

KAUN, K. R.; DEVINENI, A. V; HEBERLEIN, U. Drosophila melanogaster as a model to study drug addiction. **Human genetics**, v. 131, n. 6, p. 959–75, jun. 2012.

LEBESTKY, T. et al. Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. **Neuron**, v. 64, n. 4, p. 522–36, 25 nov. 2009.

LEE, M. J. et al. Dietary hempseed meal intake increases body growth and shortens the larval stage via the upregulation of cell growth and sterol levels in Drosophila melanogaster. **Molecules and Cells**, v. 30, n. 1, p. 29–36, 14 jul. 2010.

LEE, M. J. et al. The effects of hempseed meal intake and linoleic acid on Drosophila models of neurodegenerative diseases and hypercholesterolemia. **Molecules and Cells**, v. 31, n. 4, p. 337–342, 10 abr. 2011.

LI, H. et al. Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. **Current Biology**, v. 10, n. 4, p. 211–214, fev. 2000.

LOGAN, T. K.; LEUKEFELD, C.; FARABEE, D. Sexual and drug use behaviors among women crack users: implications for prevention. **AIDS education and prevention : official publication of the International Society for AIDS Education**, v. 10, n. 4, p. 327–40, ago. 1998.

LYNCH, W. J. et al. Animal models of substance abuse and addiction: implications for science, animal welfare, and society. **Comparative medicine**, v. 60, n. 3, p. 177–88, jun. 2010.

MCLELLAN, A. T. Substance Misuse and Substance use Disorders: Why do they Matter in Healthcare? **Transactions of the American Clinical and Climatological Association**, v. 128, p. 112–130, 2017.

MEDEIROS, K. T.; MACIEL, S. C.; SOUSA, P. F. DE. A Mulher no Contexto das Drogas: Representações Sociais de Usuárias em Tratamento. **Paidéia (Ribeirão Preto)**, v. 27, n. suppl 1, p. 439–447, 2017.

MODERNEL XAVIER, D. et al. Use of crack in pregnancy: repercussions for the newborn. **Investigacion y educacion en enfermeria**, v. 35, n. 3, p. X, out. 2017.

MORRIS, M. et al. Developmental nicotine exposure affects larval brain size and the adult dopaminergic system of Drosophila melanogaster. **BMC developmental biology**, v. 18, n. 1, p. 13, 2018.

NARKOWICZ, S. et al. Prenatal exposure to substance of abuse: a worldwide problem. **Environment international**, v. 54, p. 141–63, abr. 2013.

PACHECO, A. L. D. et al. Maternal crack cocaine use in rats leads to depressive- and anxiety-like behavior, memory impairment, and increased seizure susceptibility in the offspring. **European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology**, v. 44, p. 34–50, 2021.

PANDEY, U. B.; NICHOLS, C. D. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. **Pharmacological reviews**, v. 63, n. 2, p. 411–36, jun. 2011.

PHILIPSEN, M. H. et al. Mass Spectrometry Imaging Shows Cocaine and Methylphenidate Have Opposite Effects on Major Lipids in Drosophila Brain. **ACS Chemical Neuroscience**, v. 9, n. 6, p. 1462–1468, 20 jun. 2018.

PRANGE, R. et al. A Drosophila model of cigarette smoke induced COPD identifies Nrf2 signaling as an expedient target for intervention. **Aging**, v. 10, n. 8, p. 2122–2135, 27 ago. 2018.

REN, J. et al. Down-regulation of Decapping Protein 2 mediates chronic nicotine exposure-

induced locomotor hyperactivity in Drosophila. **PloS one**, v. 7, n. 12, p. e52521, 2012.

SILVA, S. A. et al. Balancing motherhood and drug addiction: the transition to parenthood of addicted mothers. **Journal of health psychology**, v. 18, n. 3, p. 359–67, mar. 2013.

SINGER, L.; FARKAS, K.; KLIEGMAN, R. Childhood medical and behavioral consequences of maternal cocaine use. **Journal of pediatric psychology**, v. 17, n. 4, p. 389–406, ago. 1992.

STONE, R. Pregnant women and substance use: fear, stigma, and barriers to care. **Health & Justice**, v. 3, n. 1, dez. 2015.

UNITED NATIONS OFFICE ON DRUGS AND CRIME. **World drug report 2021**. [s.l.] United Nations publication, [s.d.].

UNODC. **World Drug Report 2022**. **United Nations publication**United Nations publication, , 2022.

VELAZQUEZ-ULLOA, N. A. A Drosophila model for developmental nicotine exposure. **PLOS ONE**, v. 12, n. 5, p. e0177710, 12 maio 2017.

WORLD HEALTH ORGANIZATION. **Drugs (psychoactive)**. Disponível em: <https://www.who.int/health-topics/drugs-psychoactive#tab=tab_1>. Acesso em: 4 nov. 2022.

ZHANG, Y. et al. Nicotine-induced acute hyperactivity is mediated by dopaminergic system in a sexually dimorphic manner. **Neuroscience**, v. 332, p. 149–59, 2016.

ZIRIN, J. et al. Large-Scale Transgenic *Drosophila* Resource Collections for Loss- and Gain-of-Function Studies. **Genetics**, v. 214, n. 4, p. 755–767, 1 abr. 2020.

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