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INSTITUTO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

EDITE SANTOS SIQUEIRA

ÓLEO *FULL SPECTRUM* DE *CANNABIS SATIVA*: EFEITOS TERAPÊUTICOS E SEXO-DEPENDENTES NAS ALTERAÇÕES COMPORTAMENTAIS E SALIVARES APÓS A EXPOSIÇÃO PRÉ-NATAL AO *CRACK* EM RATOS *WISTAR*

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Defesa de mestrado apresentada ao Programa de Pós-graduação em Ciências da Saúde, da Universidade Federal de Alagoas, como requisito para obtenção do título de mestre em Ciências da Saúde.

Orientador: Prof. Dr. Olagide Wagner de Castro

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RESUMO

O uso de drogas psicoativas, como o crack, representa um grave problema de saúde pública no Brasil, afetando cerca de 2 milhões de pessoas. Seu efeito altamente viciante ocorre devido à rápida absorção nos pulmões, levando à fissura dos usuários. O impacto da droga se torna ainda mais preocupante quando consideramos as gestantes usuárias, uma vez que os metabólitos do crack atravessam a barreira placentária e causam danos no desenvolvimento fetal. Esses danos incluem déficits cognitivos, alterações comportamentais, maior risco de epilepsia, além de lesões no sistema nervoso central, frequentemente associadas à neurotoxicidade e ao estresse oxidativo. Embora existam intervenções psicossociais para o tratamento, ainda não existem terapias farmacológicas eficazes para minimizar os danos, o que torna urgente a busca por novas abordagens. Este estudo investigou o potencial terapêutico do óleo *full spectrum* de *Cannabis sativa* rico em canabidiol (FS-CBD) para reduzir os danos causados pela exposição pré-natal ao *crack* (PN-Crack). Ratas prenhes foram expostas ao *crack* (200 mg) diariamente do 5º ao 21º dia de gestação, e seus filhotes, na fase adulta, foram divididos em quatro grupos experimentais e tratados com FS-CBD (30 mg/kg, via oral) ou solução salina 0,9% por 7 dias. Foram realizados testes comportamentais para avaliar ansiedade, depressão, atividade locomotora e memória de longo prazo. Além disso, amostras de saliva, glândulas salivares, timo e baço foram coletadas para análise de peso relativo e celularidade. Os resultados revelaram, pela primeira vez, que a exposição ao PN-Crack induz alterações dependentes do sexo em comportamento, fisiologia e funções glandulares em ratos F1 adultos. As fêmeas expostas ao PN-Crack apresentaram redução no ganho de peso, sugerindo alterações metabólicas e hormonais, enquanto os machos mostraram maior resistência a essas mudanças, indicando uma resposta diferencial entre os sexos. A exposição ao PN-Crack resultou em comportamento do tipo ansioso e depressivo em ambos os sexos, sendo que o tratamento com FS-CBD foi eficaz para mitigar parcialmente esses efeitos. No que diz respeito à função salivar, observou-se que a exposição ao PN-Crack afetou o fluxo salivar e o peso glandular de maneira sexo-dependente, com o FS-CBD apresentando efeito protetor, também variando entre os sexos. Em relação aos órgãos linfoides, não foram observadas diferenças estatísticas significativas. Este estudo indica que o FS-CBD pode ser uma abordagem terapêutica promissora, especialmente considerando as diferenças sexuais, com potencial para melhorar a qualidade de vida de crianças expostas ao *crack* durante a gestação e oferecer alternativas terapêuticas inovadoras.

Palavras-chave: Canabidiol; Terapia alternativa; Comorbidades; Drogas psicoativas.

ABSTRACT

The use of psychoactive drugs, such as crack cocaine, represents a serious public health issue in Brazil, affecting approximately 2 million people. Its highly addictive effect occurs due to rapid absorption in the lungs, leading to user dependence. The impact of the drug becomes even more concerning when considering pregnant users, as crack metabolites cross the placental barrier and cause harm to fetal development. These damages include cognitive deficits, behavioral changes, increased risk of epilepsy, and lesions in the central nervous system, frequently associated with neurotoxicity and oxidative stress. Although there are psychosocial interventions for treatment, there are still no effective pharmacological therapies to minimize the damage, making the search for new approaches urgent. This study investigated the therapeutic potential of full-spectrum *Cannabis sativa* oil rich in cannabidiol (FS-CBD) to reduce the damage caused by prenatal crack cocaine exposure (PN-Crack). Pregnant rats were exposed to crack (200 mg) daily from days 5 to 21 of gestation, and their offspring, in adulthood, were divided into four experimental groups and treated with FS-CBD (30 mg/kg, orally) or saline solution (0.9%) for 7 days. Behavioral tests were conducted to assess anxiety, depression, locomotor activity, and long-term memory. Additionally, samples of saliva, salivary glands, thymus, and spleen were collected for analysis of relative weight and cellularity. The results revealed, for the first time, that exposure to PN-Crack induces sex-dependent changes in behavior, physiology, and glandular functions in adult F1 rats. Females exposed to PN-Crack showed reduced weight gain, suggesting metabolic and hormonal changes, while males showed greater resistance to these changes, indicating a differential response between sexes. Exposure to PN-Crack resulted in anxiety-like and depression-like behavior in both sexes, with FS-CBD treatment being effective in partially mitigating these effects. Regarding salivary function, PN-Crack exposure affected salivary flow and glandular weight in a sex-dependent manner, with FS-CBD showing a protective effect, also varying between sexes. No significant statistical differences were observed in the lymphoid organs. This study suggests that FS-CBD may be a promising therapeutic approach, especially considering the sex differences, with potential to improve the quality of life for children exposed to crack during gestation and offer innovative therapeutic alternatives.

Keywords: Cannabidiol; Alternative therapy; Comorbidities; Psychoactive drugs.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

µg - Microgram

µL – Microliter

ANOVA - Analysis of Variance

ANVISA - National Health Surveillance Agency

CAPES - Coordination for the Improvement of Higher Education Personnel

CB - Cannabinoid Receptor

FS-CBD - Full Spectrum *C. sativa* Oil, rich in cannabidiol

CEMIB - Multidisciplinary Center for Biological Research

CEUA - Ethics Committee on the Use of Animals

CNPq - National Council for Scientific and Technological Development

CNS - Central Nervous System

CONCEA - National Council for Animal Experimentation Control

CRK - Crack Cocaine

CTRL - Control

DI - Discrimination Index

EOA - Entries in Open Arms

EPI - Personal Protective Equipment

EPM - Elevated Plus Maze

FAPEAL - Foundation for the Support of Research in the State of Alagoas

FST- Forced Swim Test

GABA - Gamma-Aminobutyric Acid

GD - Gestational Day

HPA - Hypothalamus-Pituitary-Adrenal Axis

I.P - Intraperitoneal

LNFI - Laboratory of Integrative Neurophysiology and Pharmacology

NaOH - Sodium Hydroxide

OF - Open Field

ORT - Object Recognition Test

P - Post-natal

PI - Preference Index

PN-Crack - Prenatal Exposure to Crack Cocaine

PPGCS - Graduate Program in Health Sciences

SECB - Endocannabinoid System

SEM - Standard Error of the Mean

THC - Tetrahydrocannabinol

TOA - Time in Open Arms

UFAL - Federal University of Alagoas

UNICAMP - State University of Campinas

VEH - Vehicle (0.9% Sodium Chloride Solution)

SUMÁRIO

1. INTRODUÇÃO

O consumo exacerbado de drogas é um problema de ordem global, afetando economicamente e socialmente diversos países. A dependência química afeta o sistema nervoso central (SNC) e modula diversos tipos de comportamento, *e.g*., agressividade (Bastos; Bertoni, 2014-). Os problemas sociais se potencializam quando os usuários apresentam comportamentos violentos e envolvimento com a prática de atividades ilícitas como roubo, tráfico e prostituição em busca da manutenção o vício (Ribeiro; Sanchez; Nappo, 2010).

O consumo de drogas tem crescido significativamente nos últimos anos. Em 2021, cerca de 296 milhões de pessoas utilizaram drogas psicotrópicas, e o uso recorrente dessas substâncias resultou na morte de quase meio milhão de pessoas, enquanto os transtornos relacionados ao uso de drogas geraram uma série de comorbidades (United Nations Office on Drugs and Crime, 2024). De forma preocupante, a produção global de cocaína também segue em um ritmo acelerado. Entre 2020 e 2021, foi registrado um aumento expressivo nas atividades de cultivo de coca, com um crescimento de 35% no período; a maior elevação anual desde 2016 (United Nations Office on Drugs and Crime, 2024). Esse aumento deve-se tanto à expansão das áreas de cultivo quanto à melhoria nos processos de conversão das folhas de coca em cloridrato de cocaína (United Nations Office on Drugs and Crime, 2024). No Brasil, aproximadamente 6 milhões de pessoas já experimentaram cocaína uma vez na vida e cerca de 2 milhões, utilizaram o seu derivado, o *crack* (Bastos; Bertoni, 2014).

O *crack* é uma forma fumada de cocaína, derivada da pasta-base misturada com bicarbonato de sódio e outros produtos químicos, o que intensifica seu potencial adictivo devido à rápida absorção pulmonar, além de baratear a sua compra (Andrade *et al.*, 2023). Esse composto tem um alto risco de dependência, caracterizado pela fissura, definida como um forte desejo compulsivo e repetitivo do seu uso (Chaves *et al.*, 2011; Leite; Andrade, 1999). A interrupção abrupta do consumo, após exposições repetidas ao *crack*, está associada a sintomas graves de abstinência, como ansiedade e depressão, devido à alteração dos sistemas dopaminérgicos e serotoninérgicos (Gawin, 1991; Silva *et al.*, 2021). Além disso, usuários de *crack* frequentemente apresentam dificuldades em aderir a tratamentos convencionais ao vício, resultando em altas taxas de abandono, especialmente na ausência de abordagens integrativas que tratem tanto a dependência quanto os transtornos psiquiátricos coexistentes (Duailibi; Ribeiro; Laranjeira, 2008).

Embora não existam medicamentos específicos aprovados exclusivamente para o tratamento da dependência química de *crack*, alguns fármacos são utilizados com o objetivo de tratar sintomas associados, como os transtornos mentais (e.g., ansiedade e depressão) e alterações nas vias dopaminérgicas, que são comuns nessa condição. Entre os fármacos mais utilizados, destacam-se a buprenorfina, a naltrexona, o topiramato, a bupropiona e a modafinila (Castells *et al.*, 2016; Karila *et al.*, 2008). O tratamento padrão ao vício de *crack* engloba abordagens psicossociais, mas em muitos casos, não se mostra totalmente efetivo e está associado a altas taxas de abandono (Kampman, 2019).

O uso recorrente de *crack* causa uma série de efeitos adversos no indivíduo, incluindo quadros febris, irritabilidade, sudorese, convulsões e vômitos, que estão frequentemente associados às alterações nos níveis cerebrais de dopamina e serotonina, caracterizando a síndrome de abstinência (Butler; Rehm; Fischer, 2017). O uso crônico dessa substância compromete o sistema imunológico, afetando a maturação das células T no timo e prejudicando a produção de linfócitos B no baço (Ersche; Döffinger, 2017). Além disso, o *crack* induz alterações hormonais, provoca inflamação crônica e reduz a eficácia geral do sistema imunológico, tornando o organismo mais vulnerável a infecções e doenças autoimunes (Mello; Mendelson, 1997).

O uso prolongado também impacta as glândulas salivares e o fluxo salivar, resultando em redução da produção de saliva devido aos efeitos vasoconstritores da droga, além de inflamação, danos e atrofia das glândulas salivares, especialmente das glândulas parótidas, comprometendo sua função protetora e aumentando o risco de infecções orais (Melo *et al.*, 2022). Com o uso continuado do *crack*, os efeitos adversos ultrapassam o indivíduo, tornando-se problemas sociais que se potencializam quando os usuários apresentam comportamentos violentos e envolvimento com a prática de atividades ilícitas como roubo, tráfico e prostituição em busca da manutenção o vício (Ribeiro; Sanchez; Nappo, 2010).

A problemática do vício do *crack* é mais alarmante em virtude de mulheres grávidas continuarem o uso da droga durante o período gestacional. Durante a gestação, naturalmente ocorre a diminuição da expressão das colinesterases plasmáticas e hepáticas, reduzindo a metabolização da droga nas gestantes e potencializando os efeitos adversos da droga (Kuczkowski, 2004; Richardson; Goldschmidt; Larkby, 2007). Esse mecanismo pode resultar em complicações tanto para a mãe quanto para o feto (Duailibi; Ribeiro; Laranjeira, 2008), uma vez que os metabólitos do *crack* atravessam a barreira placentária e promovem efeitos prolongados em diferentes etapas do desenvolvimento fetal (Delagneau *et al.*, 2023; Ganapathy, 2011).

Efeitos adversos *in utero*, como prematuridade, baixo peso ao nascer, restrição do crescimento intrauterino, comprometimento do desenvolvimento neurológico, malformações congênitas, aumento do risco de aborto espontâneo e síndrome da abstinência neonatal, o potencial adverso do *crack* pode perdurar na primeira infância e vida adulta dos filhos de usuárias (Arendt; Minnes; Singer, 1996; Kessler; Pechansky, 2008b; Legido *et al.*, 1992; Silveira *et al.*, 2020). Em geral, estas crianças não respondem organizadamente aos estímulos ambientais e apresentam sintomas da síndrome de abstinência, déficits neuropsicomotores e cognitivos de longo prazo, incluindo: falha no desenvolvimento de linguagem, aprendizado e memória, raciocínio perceptivo e problemas comportamentais (Kessler; Pechansky, 2008a; Legido *et al.*, 1992).

Adicionalmente, os efeitos da cocaína/*crack* em crianças estão associados a déficits cognitivos, dificuldade na verbalização, agressividade, depressão (Bandstra *et al.*, 2010; van Baar *et al.*, 1994) e a susceptibilidade para crises epilépticas na fase adulta (Slamberová, 2003). Corroborando isso, nosso grupo de pesquisa demonstrou que a exposição ao *crack* durante o período gestacional de ratas *Wistar* induziu alterações comportamentais (fenótipo ansiogênico e depressivo) e cognitivas (déficits na memória), assim como, aumento da gravidade de crises epilépticas induzidas por modelo de pilocarpina e neurodegeneração cerebral (*e.g.*, em núcleos amigdaloides e hipocampo) na prole (Pacheco *et al.*, 2021). Considerando esses efeitos graves, torna-se evidente a necessidade de desenvolver terapias alternativas mais eficazes e seguras para tratar as comorbidades associadas ao consumo de crack.

Diante da limitação dessas abordagens, as terapias alternativas têm ganhado destaque como potenciais soluções para mitigar os efeitos do crack e outras drogas psicoativas. Vários psicomiméticos vêm sendo utilizados como uma ferramenta alternativa para a superação de dependência de drogas de abuso e tratamento de comorbidades associadas, tais como: a maconha *Cannabis sativa* (García-Gutiérrez *et al.*, 2020; Kaplan *et al.*, 2017), o peyote *Lophophorawilliansii* (Halpern *et al.*, 2005; Valdez *et al.*, 2024), a iboga *Tabernanthe iboga*, (Gomes, 2021; Rocha *et al.*, 2023), e a ayahuasca (James *et al.*, 2022; Rocha *et al.*, 2023).

A *C. sativa* é uma das plantas mais pesquisadas atualmente pelo seu potencial terapêutico em distúrbios neurológicos (Atalay; Jarocka-Karpowicz; Skrzydlewska, 2019; Ross-Munro; Isikgel; Fleiss, 2024; Vitale; Iannotti; Amodeo, 2021). Em modelos animais, estudos revelam que canabinoides variados possuem propriedades ansiolíticas, antidepressivas, antipsicóticas, antiepiléticas, neuroprotetoras, anti-inflamatórias e antioxidantes, indicando amplas aplicações terapêuticas para diversos transtornos

psiquiátricos, neurológicos e aqueles relacionados ao uso de substâncias psicoativas (García-Gutiérrez *et al.*, 2020; Pertwee, 2009).

O sistema endocanabinoide (SECB) desempenha um papel central na regulação de processos fisiológicos e emocionais como humor, estresse, memória e controle motor (Melo Reis *et al.*, 2021). Essa rede moduladora é composta receptores endocanabinoides (CB1 e CB2), e enzimas envolvidas na síntese e degradação de seus ligantes. Além dos endocanabinoides, fitocanabinoides encontrados na *C. sativa*, como canabidiol (CBD) e tetrahidrocanabidiol (THC) ativam o SECB influenciando diversas funções neurobiológicas (Rezende *et al.*, 2023). O SECB modula neurotransmissores como serotonina, GABA, dopamina e glutamato, contribuindo para efeitos ansiolíticos e antidepressivos (García-Gutiérrez et al., 2020). Além disso, regula o eixo hipotálamo-pituitária-adrenal (HPA), influenciando a resposta ao estresse e a homeostase emocional (Osborne et al., 2019). Nas glândulas salivares, a ativação do CB1 pode reduzir a liberação excessiva de noradrenalina pelos terminais nervosos simpáticos, atenuando a hiposalivação induzida por drogas como o *crack*, o CB2, por sua vez, exerce efeitos anti-inflamatórios e regenerativos, promovendo a recuperação da função secretora e minimizando danos teciduais (Antoniazzi *et al.*, 2017; Busch; Sterin-Borda; Borda, 2004).

Apesar do exposto acima, estudos sobre o efeito protetor do canabidiol em comorbidades associadas a processos neurodegenerativos em adultos expostos à fumaça do *crack* durante a gestação, ainda são escassos, mas podem abrir caminhos para novas estratégias de tratamento. Uma terapia alternativa pode oferecer avanços científicos importantes para melhorar a sobrevivência e qualidade de vida de adultos expostos a essa substância durante o período gestacional. Neste estudo, testamos a hipótese de que o tratamento com óleo *full spectrum* de *C. sativa*, rico em canabidiol, reduz as comorbidades associadas à exposição pré-natal à fumaça do *crack*, promovendo melhorias no comportamento, em parâmetros fisiológicos e função glandular da prole adulta (F1).

2. OBJETIVO

2.1 Objetivo geral

Elucidar o efeito do tratamento com o óleo *full spectrum* de *C. sativa* rico em canabidiol (FS-CBD) na redução de comorbidades associadas à exposição à fumaça do crack, durante o período gestacional, incluindo possíveis alterações nos órgãos linfoides e glândulas salivares na prole adulta (F1).

2.2 Objetivos específicos

- Investigar os efeitos do tratamento com FS-CBD sobre comportamentos do tipo ansioso e a atividade locomotora de animais (F1) expostos aos produtos da pirólise do *crack* durante o período pré-natal;
- Analisar o impacto do tratamento com FS-CBD no comportamento do tipo depressivo em animais (F1) expostos aos produtos da pirólise do crack durante o período pré-natal;
- Avaliar o efeito do tratamento com FS-CBD na consolidação da memória m animais (F1) expostos aos produtos da pirólise do *crack* durante o período pré-natal;
- Verificar os efeitos da exposição ao *crack* e do tratamento com FS-CBD sobre o peso relativo e a celularidade do timo e baço;
- Avaliar o efeito do tratamento com FS-CBD sobre as glândulas salivares e o fluxo de salivar em animais (F1) expostos aos produtos da pirólise do *crack* durante o período pré-natal.

Artigo. *Full spectrum Cannabis sativa oil: therapeutic and sex-dependent effects on behavioral and salivary changes after prenatal exposure to crack cocaine in rats*

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ABSTRACT

The use of psychoactive drugs, such as crack cocaine, represents a serious public health issue in Brazil, affecting approximately 2 million people. Its highly addictive effect occurs due to rapid absorption in the lungs, leading to user dependence. The impact of the drug becomes even more concerning when considering pregnant users, as crack metabolites cross the placental barrier and cause harm to fetal development. These damages include cognitive deficits, behavioral changes, increased risk of epilepsy, and lesions in the central nervous system, frequently associated with neurotoxicity and oxidative stress. Although there are psychosocial interventions for treatment, there are still no effective pharmacological therapies to minimize the damage, making the search for new approaches urgent. This study investigated the therapeutic potential of full-spectrum *Cannabis sativa* oil rich in cannabidiol (FS-CBD) to reduce the damage caused by prenatal crack cocaine exposure (PN-Crack). Pregnant rats were exposed to crack (200 mg) daily from days 5 to 21 of gestation, and their offspring, in adulthood, were divided into four experimental groups and treated with FS-CBD (30 mg/kg, orally) or saline solution (0.9%) for 7 days. Behavioral tests were conducted to assess anxiety, depression, locomotor activity, and long-term memory. Additionally, samples of saliva, salivary glands, thymus, and spleen were collected for analysis of relative weight and cellularity. The results revealed, for the first time, that exposure to PN-Crack induces sex-dependent changes in behavior, physiology, and glandular functions in adult F1 rats. Females exposed to PN-Crack showed reduced weight gain, suggesting metabolic and hormonal changes, while males showed greater resistance to these changes, indicating a differential response between sexes. Exposure to PN-Crack resulted in anxiety-like and depression-like behavior in both sexes, with FS-CBD treatment being effective in partially mitigating these effects. Regarding salivary function, PN-Crack exposure affected salivary flow and glandular weight in a sex-dependent manner, with FS-CBD showing a protective effect, also varying between sexes. No significant statistical differences were observed in the lymphoid organs. This study suggests that FS-CBD may be a promising therapeutic approach, especially considering the sex differences, with potential to improve the quality of life for children exposed to crack during gestation and offer innovative therapeutic alternatives.

Keywords: Cannabidiol; Alternative therapy; Comorbidities; Psychoactive drugs.

1. INTRODUCTION

The use of psychoactive drugs represents a global public health issue with significant economic, social, and healthcare impacts (United Nations Office on Drugs and Crime, 2024). In Brazil, it is estimated that approximately 6 million people have used cocaine at least once in their lifetime, and there are around 2 million individuals dependent on crack, a highly addictive derivative (Bastos; Bertoni, 2014). Crack cocaine is smoked and rapidly absorbed through the lungs, displaying a high addictive potential. It induces intense craving in users, characterized by a compulsive and uncontrollable desire for use, as well as symptoms such as anxiety, depression, and severe withdrawal seizures (Chaves *et al.*, 2011).

Although efforts have been made to treat crack cocaine dependence, primarily through psychosocial approaches, the high dropout rates limit the effectiveness of these conventional strategies (Duailibi; Ribeiro; Laranjeira, 2008; Kampman, 2019). The scenario is even more concerning in pregnant users, as crack cocaine metabolites cross the placental barrier, compromising intrauterine development and significantly affecting the health and lifelong learning potential of the offspring (Slamberová, 2003). Additionally, cognitive deficits, behavioral changes, and increased susceptibility to epileptic seizures are associated with drug exposure during intrauterine life (Ganapathy, 2011; Kessler; Pechansky, 2008a).

Preclinical studies conducted by our research group, using an animal model, confirm the severity of prenatal crack cocaine exposure, demonstrating that its consumption during pregnancy leads to behavioral changes, memory deficits, and neurodegeneration in brain structures crucial to the limbic system (Pacheco *et al.*, 2021). These findings highlight the urgency of investigating new therapeutic strategies for the comorbidities associated with crack cocaine addiction, as the currently available alternatives prove insufficient to address the complexity of the damage caused by this psychoactive substance.

In this context, *Cannabis sativa* L. (1753) has garnered increasing interest within the scientific community due to its neuroprotective, anxiolytic, and anti-inflammatory properties (Atalay; Jarocka-Karpowicz; Skrzydlewska, 2019; García-Gutiérrez et al., 2020). Its bioactivity can modulate the endocannabinoid system (ECS) by binding to the endocannabinoid receptors (CB1 and CB2). Once activated, the ECS plays a central role in regulating mood, memory, and stress response. The ECS has the potential to attenuate neurological and psychiatric deficits associated with the use of psychoactive substances such as crack cocaine (Melo Reis et al., 2021; Osborne et al., 2019). CB1 and CB2 receptors are expressed not only in the central nervous system but also in peripheral organs, including the

thymus, spleen, and salivary glands, where they contribute to immune modulation, inflammatory responses, and homeostasis. However, studies demonstrating the effects of CBD concerning comorbidities induced by psychoactive substances, such as crack cocaine, are scarce, particularly regarding its impact on these peripheral structures.

The potential medicinal use of *C. sativa* emerges as a promising therapeutic alternative to mitigate the damage caused by crack cocaine exposure in adults during pregnancy. This article aims to analyze the use of full-spectrum *C. sativa* oil, rich in cannabidiol, in the treatment of comorbidities associated with crack cocaine consumption during pregnancy, highlighting its therapeutic potential and the challenges in implementing this approach. In this study, we test the hypothesis that treatment with full-spectrum *C. sativa* oil, rich in cannabidiol, reduces comorbidities associated with prenatal exposure to crack cocaine smoke, promoting improvements in behavior, physiological parameters, and glandular and lymphoid organ function in the adult offspring (F1).

2. METHODOLOGY

2.1 Animals

Our study was conducted following the guidelines established by the Animal Research Report: In Vivo and approved by the Ethics Committee of the Federal University of Alagoas (UFAL; CEUA: protocol number #28/2021; **Annex 1**). Wistar rats (females; n=30; males; n=15) were obtained from the Multidisciplinary Center for Biological Research at the State University of Campinas of Brazil (CEMIB-UNICAMP). The animals were acclimated and housed in the animal facility of the Laboratory of Neuropharmacology and Integrative Physiology (LNFI-UFAL) and divided into groups of five animals per cage, under controlled temperature, submitted to a 12-hour light/dark cycle (lights on at 07:00), with water and food available *ad libitum*.

2.2 Drugs

2.2.1 Crack cocaine

The crack cocaine samples were previously obtained through the Civil Police of the State of Alagoas (AL-Brazil), with authorization from the National Health Surveillance Agency (ANVISA) and a court order mandating the delivery and storage of the substance in our laboratory (LNFI—UFAL). The Federal Attorney's Office based at UFAL organized all documentation, following a request from the esteemed Rector.

2.2.2 Full-spectrum *Cannabis sativa* oil

We used a concentration of 30 mg/kg of full-spectrum *C. sativa* oil rich in cannabidiol (FS-CBD), purchased through the Arapiraquense Association of Medicinal Cannabis Patients (REGENERA). Analyses of the oil revealed a total cannabinoid content of 85.23%, with 76.21% cannabidiol and 2.32% tetrahydrocannabinol (**Annex 2**). The treatment concentration was selected based on doses previously used in other experimental models with rodents (Moore; Weerts, 2022; Mottarlini *et al.*, 2022; Shbiro *et al.*, 2019a).

2.3 Prenatal exposure to crack cocaine (PN-Crack)

The animals were mated following the protocol previously published by our group in Pacheco et al. (2021). Briefly, when the presence of spermatozoa was observed after vaginal smears in mated females, it was considered as day 0 of pregnancy (GD 0). Subsequently, with pregnancy confirmation, the females were individually housed.

The pregnant rats were randomized into two treatment groups: the crack-exposed group (PN-Crack) and the control group (exposed to ambient air). Exposure to air for the control group mimicked the environmental and handling conditions of the crack cocaine-exposed group. The maternal body weight of all rats (control and exposed) was measured to ensure that there was no anorexigenic effect characteristic of the cocaine present in the crack samples.

The animals were exposed to crack cocaine pyrolysis through an adapted apparatus used for cigarette smoking. The system routinely employed in our laboratory (Pacheco *et al.*, 2021), consists of a smoke-generating pump connected at one end to a pipe and at the other to an acrylic chamber (where the animals are housed). The combustion content (smoke) is propelled by a rotor configured to generate 150 ml/min. The entire experiment was conducted in a designated exposure room equipped with a hood containing a filter, specific exhaust fans, and the necessary personal protective equipment (PPE).

For each experiment, the pregnant rats were exposed to 200 mg of crack cocaine daily and individually from the 5th to the 21st gestational day (GD). In contrast, the control group was exposed only to ambient air within the hood for 10 minutes. After the offspring were born, considered on postnatal day (P0), all litters were adjusted to eight pups to ensure that each mother had the same number of pups (4 males and 4 females). All animals were weaned at P20 and housed to form the following groups: a) CTRL+VEH [control animals

treated with 0.9% saline solution], b) CTRL+FS-CBD [control animals treated with full-spectrum C. sativa oil rich in cannabidiol], c) CRK+VEH [exposed animals treated with 0.9% saline solution]; d) CRK+FS-CBD [exposed animals treated with full-spectrum *C. sativa* oil rich in cannabidiol] until they reached puberty. The number of animals per group was 8 to 10, depending on exclusions during the experimental process.

2.4 Treatment

The animals were treated with full-spectrum *Cannabis sativa* oil rich in cannabidiol (FS-CBD; 30mg/kg) or with saline solution (VEH; NaCl 0.9%), administered once a day for 7 days, between P53 and P60. The animals were gently restrained and received oral administration of FS-CBD or VEH via a pipette directly into the mouth. The procedure was performed daily at 9 a.m. under the same laboratory conditions. All animals were weighed before administration to monitor weight throughout the treatment.

2.5 Behavioral analysis

All behavioral experiments were conducted between P60 and P65 during the afternoon (from 12:00 p.m. to 6:00 p.m.). The same researcher placed these animals individually in the behavioral apparatus to avoid the influence of different handling techniques. A camera (Intelbras®, Santa Catarina, Brazil; model VD 3108) was positioned approximately 1 meter above the behavioral apparatus to record and later observe the animal behaviors.

2.5.1 Behavioral test to assess fear and anxiety-like behavior

The Elevated Plus Maze (EPM; Insight®, São Paulo, Brazil) test consists of two open arms (30 x 5 x 25 cm) and two closed arms (30 x 5 x 25 cm), arranged in a cross shape and elevated 40 cm above the floor. The animals were submitted to the EPM, placed individually at the central platform of the apparatus, with their head facing one of the closed arms, and their behavior was evaluated for 5 minutes. The percentage of time spent and the number of entries into the open and closed arms were assessed. Additionally, the following ethological measures were evaluated: protective stretched attend-posture, unprotected head-dipping, grooming, and rearing (Blanchard; Blanchard, 1989). At the end of each evaluation period, each animal's EPM was cleaned with 10% alcohol.

2.5.2 Behavioral test to assess locomotion

The Open Field Test (OF, Insight®, São Paulo, Brazil; 50 cm height x 90 cm diameter) allows the assessment of the animal's spontaneous locomotor activity. The OF consists of a circular acrylic arena, with the floor divided into eight sections. The animal was placed at the center of the apparatus, and the number of crossings between the lines of the apparatus and the amount of rearing was counted over a 5-minute. A reduction in the number of crossings, with all four paws, between the divisions of the field, when compared to the control group, may be interpreted as impairment in spontaneous locomotor activity. On the other hand, an increase in this parameter may be interpreted as an improvement in motor performance (Kraeuter; Guest; Sarnyai, 2019). The OF was performed immediately after the EPM.

2.5.3 Behavioral test to assess depression-like behavior

The Forced Swim Test (FST), initially proposed by Porsolt; Le Pichon; Jalfre, (1977), consists of two phases: pre-test and test. The rodents were placed in an aversive situation, swimming in a cylindrical tank filled with water (22x22x32 cm) at the recommended temperature (25-26°C Pacheco *et al*., 2021), where there is no possibility of escape. Animals that remained immobile for more prolonged periods were considered to exhibit depressive-like behavior (Slattery; Cryan, 2012). The pre-test duration is 15 minutes on the first day, during which the animal begins to move to keep its head above water. On the test day (24 hours after the pre-test), the animal was placed back into the water cylinder and remained in the test for 5 minutes. The parameters analyzed were latency to immobility and time spent immobile (Mu *et al.*, 2020; Porsolt, 1977). After the test, the animal was placed in a small box with paper towels and heated with a lamp until its fur was completely dry.

2.5.4 Behavioral test to assess memory

The Object Recognition Test (ORT) was used to assess long-term memory and was divided into two phases: training and testing. During the training phase, animals were placed in the behavioral arena (the same as in the OF) with two identical objects positioned at pre-established distances (Antunes; Biala, 2012). After 24 hours, the animal was placed back in the arena, but one of the objects was replaced by a new object with a different color, shape, and size compared to the familiar object. The parameters analyzed were the discrimination Index (DI) and the Preference Index (PI) (Antunes; Biala, 2012). The DI allows for discrimination between the new and familiar objects, that is, it uses the difference in time spent exploring the familiar object (TF) and then divides that value by the total exploration

time of the new (TN) and familiar objects: DI = (TN − TF) / (TN + TF). The PI is the proportion of time spent exploring either of the two objects: PI = TN / (TN + TF) × 100 (%). A PI above 50% indicates a preference for the new object, below 50% a preference for the familiar object, and equal to 50% indicates no preference (Hammond; Tull; Stackman, 2004). The apparatus was cleaned with 10% alcohol between animals tests.

2.6 Salivary gland collection and salivary flow evaluation

Before euthanasia, the animals were anesthetized with sodium thiopental administered intraperitoneally (60 mg/kg, i.p.). Then, pilocarpine (2 mg/kg, i.p., a cholinergic agent) was used to stimulate salivary secretion. Saliva collection was performed for 10 minutes, using a pipette for collection, starting immediately after observing the onset of salivation, as described by Melo et al. (2020). The volume of saliva was determined by the weight difference of an Eppendorf microtube before and after collection, using a precision analytical balance (PSC AUW220 Shimadzu©).

After euthanasia, the major salivary glands (parotid and submandibular, right and left) were carefully dissected and weighed individually. The salivary flow ratio was calculated by adding the weight of the right submandibular gland to the right parotid gland and dividing this value by the total volume of saliva collected (Melo *et al.*, 2020). The formula used was:

$$Salivary\ flow\ (g/\mu L) = \frac{Weight\ (Sub.D + Par.D)(g)}{Vol.\ salivary\ (\mu L)}$$

2.7 Euthanasia

The animals were euthanized by decapitation, 24 hours after the last behavioral test evaluation. For euthanasia, specific equipment for this purpose was used (Guillotine - Bonther ©), following the Guidelines for the Practice of Euthanasia of the National Council for Animal Experimentation Control (CONCEA), according to RN 37/2018.

2.8 Collection and evaluation of immune organs

After euthanasia of the offspring, the main immune organs, thymus and spleen, were extracted, weighed, and physically disrupted in phosphate-buffered saline (PBS) with 2% fetal bovine serum (FBS) (both from Invitrogen, Carlsbad, CA, USA) in a 24-well plate. Then, the thymocytes and splenocytes in suspension were counted in a Neubauer chamber using the exclusion method with 0.02% Trypan blue solution (Sigma-Aldrich, St. Louis,

MO, USA). Turk's solution was used for the lysis of red blood cells from the spleen (composed of 1% glacial acetic acid and 0.1% crystal violet – Dinâmica Química, Indaiatuba, SP, Brazil – in distilled water). The **Figure 1** presents a summary of the steps described in the methodology, visually representing the sequence of experiments as detailed in the text.

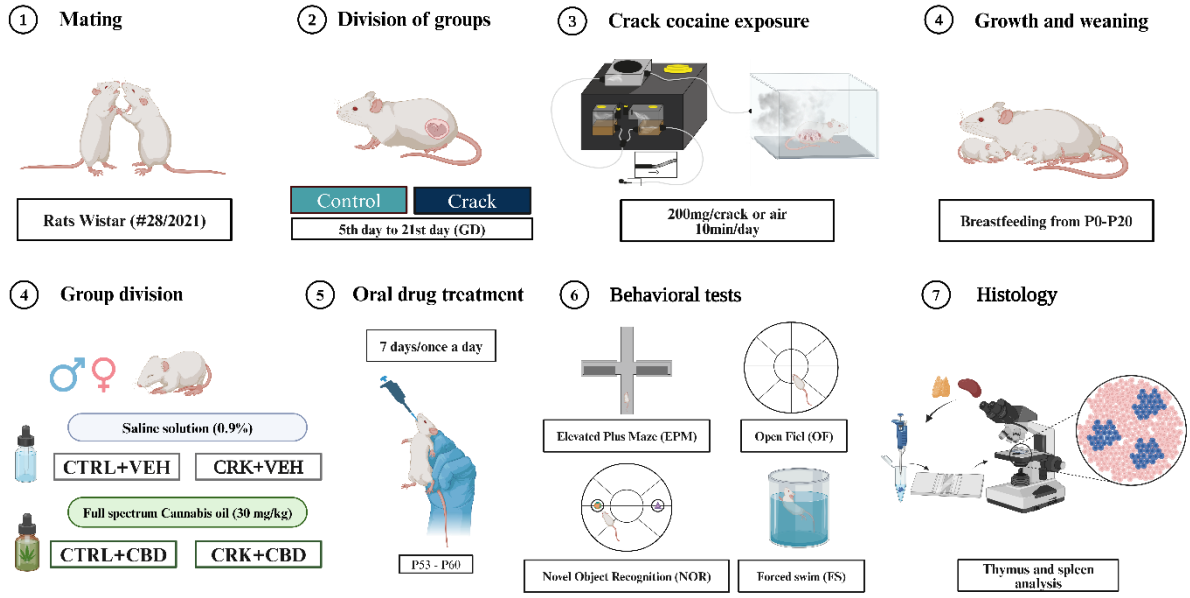

Figure 1. Experimental Design. Schematic representation of exposure to crack cocaine, treatment, and the experiments conducted. Demonstration of the crack cocaine exposure apparatus adapted from Pacheco et al. (2021) and BioRender.com model (2024). Obtained from https://app.biorender.com/biorender.

2.9 Statistical analyses

All experimental data were described as mean ± Standard Error Mean (SEM), assuming a significance level of 5% ($p < 0.05$) for all statistical tests using the GraphPad Prism program (version 8.00 for Windows, GraphPad Software, San Diego, CA, USA). Comparisons of results were performed using the unpaired t-test or three-way ANOVA followed by the Tukey post-hoc test. For comparison, the data were evaluated for normality and variances.

3. RESULTS

3.1 Effect of PN-Crack exposure and FS-CBD treatment on offspring weight

The animals were treated for 7 days and during this period all were weighed daily to assess weight gain or loss in response to FS-CBD treatment. The results showed that, among females, exposure to PN-Crack as well as treatment with FS-CBD led to a reduction in weight gain compared to the control group throughout the observed period (CTRL+CBD vs CTRL+VEH; CRK+VEH vs CTRL+VEH; CRK+FS-CBD; $p>0.05$, **Figure 2A**). In contrast, no significant difference was detected between male groups, suggesting a sex-dependent response to treatment (**Figure 2B**).

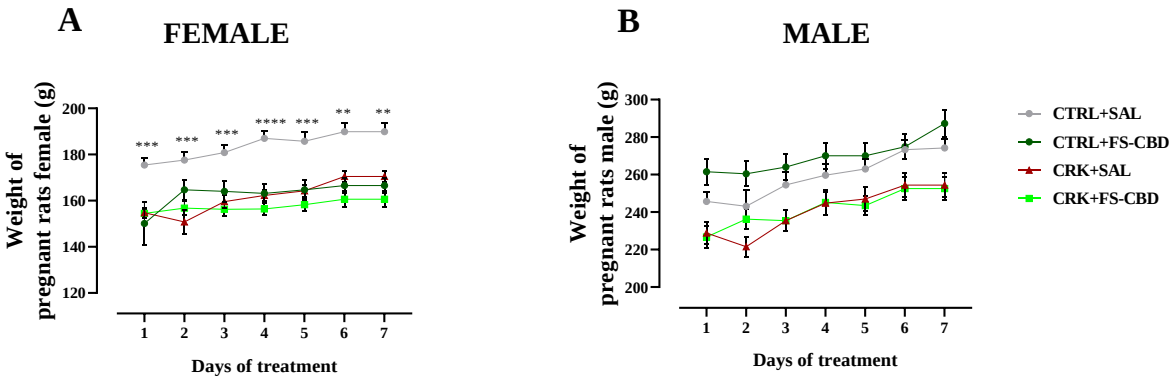

Figure 2. Effect of PN-Crack exposure and FS-CBD treatment on offspring's average body weight Female exposed to PN-Crack and treated with FS-CBD showed a lower mean body weight gain compared to the control group (A). No significant differences were observed between the studied groups for males (B). The data represents the mean ± SEM of 8-10 rats per group. *$p<0.05$, compared to control; three-way ANOVA followed by Tukey's post-hoc test. CRK, crack; FS-CBD, full spectrum *Cannabis sativa* oil rich in cannabidiol; CTRL, control; VEH, vehicle.

3.2 Effects of FS-CBD treatment on anxiety-like behavior after PN-Crack exposure

Data previously published by our research group (Pacheco et al., 2021) reveal that PN-Crack exposure induces anxiogenic-like behavior in P30 animals in the EPM. Therefore, we wanted to evaluate whether this profile persisted in adult animals, specifically at P60. After performing the EPM **(Figure 3A)**, it was observed that PN-Crack exposure significantly reduced the percentage of time spent in the open arms (%TBA) for both female and male rats in CTRL+VEH vs CRK+VEH (females, $p=0.03$; males, $p=0.05$), indicating an anxiogenic-like behavior. FS-CBD treatment showed a trend towards increased %TBA

only in female animals after crack exposure (CRK+FS-CBD vs CRK+VEH, females; p = 0.06), suggesting an anxiolytic-like profile **(Figure 3B)**. As for the percentage of entries into the open arms (%EBA) in the EPM, no significant changes (p>0.05) were observed in the groups exposed to PN-Crack and/or treated with FS-CBD (females and males) compared to CTRL+VEH or CRK+VEH **(Figure 3C)**.

Regarding the ethological parameters, also evaluated in the EPM, regarding the stretching-attending posture parameter, which is related to risk assessment behavior, a significant reduction was observed in females exposed to PN-Crack and treated with FS-CBD compared to females in the group exposed only to crack and not treated with FS-CBD (CRK+VEH vs CRK+FS-CBD, females; p=0.02) **(Figure 3D)**. On the other hand, no significant differences were observed in the rearing and cleaning parameters (grooming), either for females or males (p>0.05) **(Figure 3E and G)**. Exposure to PN-Crack in females decreased head tilt behavior compared to the control group (CTRL+VEH vs CRK+VEH, females; p=0.03). However, this was not observed in male animals. FS-CBD treatment did not significantly influence the parameters evaluated in either sex (p>0.05) **(Figure 3F)**.

PN-Crack exposure induced a persistent anxiogenic-like behavior in adulthood (P60) in both sexes. FS-CBD treatment exhibited a potentially anxiolytic effect only in females, showing a trend toward increased time in the open arms and a significant reduction in risk assessment behavior.

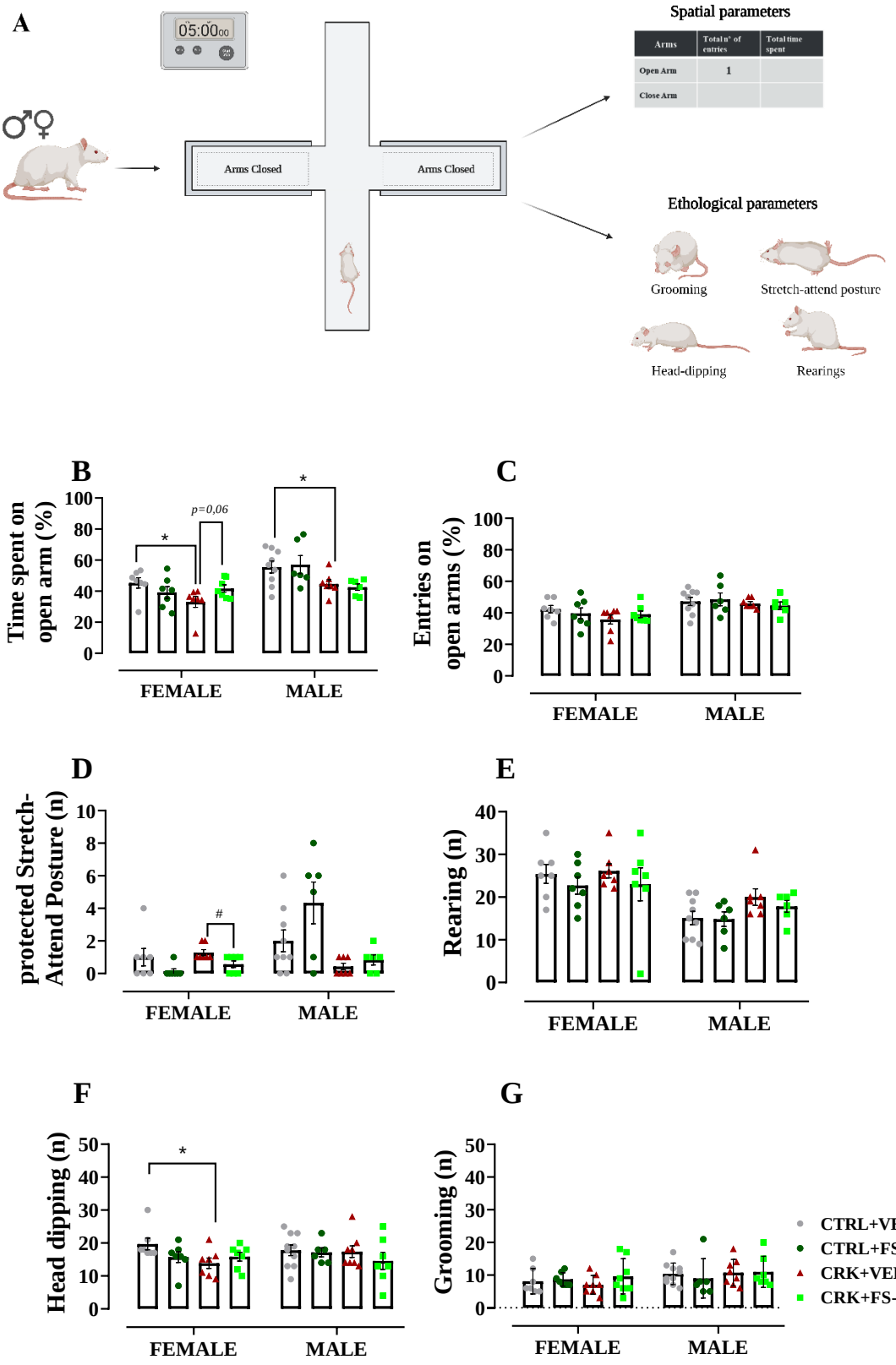

Figure 3. Effects of PN-crack exposure on anxiety-like behavior evaluated by the elevated plus maze (EPM) test. Schematic representation of the EPM test methodology (A). PN-Crack exposure reduced the percentage of time in the open arms in both sexes, while FS-CBD treatment increased this parameter only in females (B).

No significant differences were observed in the number of entries into the open arms (C), *rearing* (E), or *grooming* (G). FS-CBD treatment reduced the number of stretched-attention postures in females (D), and PN-Crack exposure decreased head lowering behavior in females, with no significant differences in males (F). *N* = 8–10 per group. $p < 0.05$ vs. CTRL+VEH, # $p < 0.05$ vs. CRK+VEH (Three-way ANOVA, Tukey's post-hoc test). CRK, crack; FS-CBD, full-spectrum *Cannabis sativa* oil rich in cannabidiol; CTRL, control; VEH, vehicle.

3.3 Effects of FS-CBD treatment on spontaneous locomotion after PN-Crack exposure

To verify whether exposure to PN-Crack and FS-CBD treatment influenced the animals' spontaneous locomotion, we conducted the open field test (OF) **(Figure 4A)**. The changes observed in the EPM were not associated with motor impairments, as the three-way ANOVA did not identify significant differences in the number of crossings or rearings in the OF between the exposure to crack cocaine (CRK+VEH; p>0,05) and FS-CBD treatment (CRK+FS-CBD; p>0,05) groups analyzed **(Figure 4B and C)**. The animals' spontaneous locomotion was not affected by crack exposure or treatment.

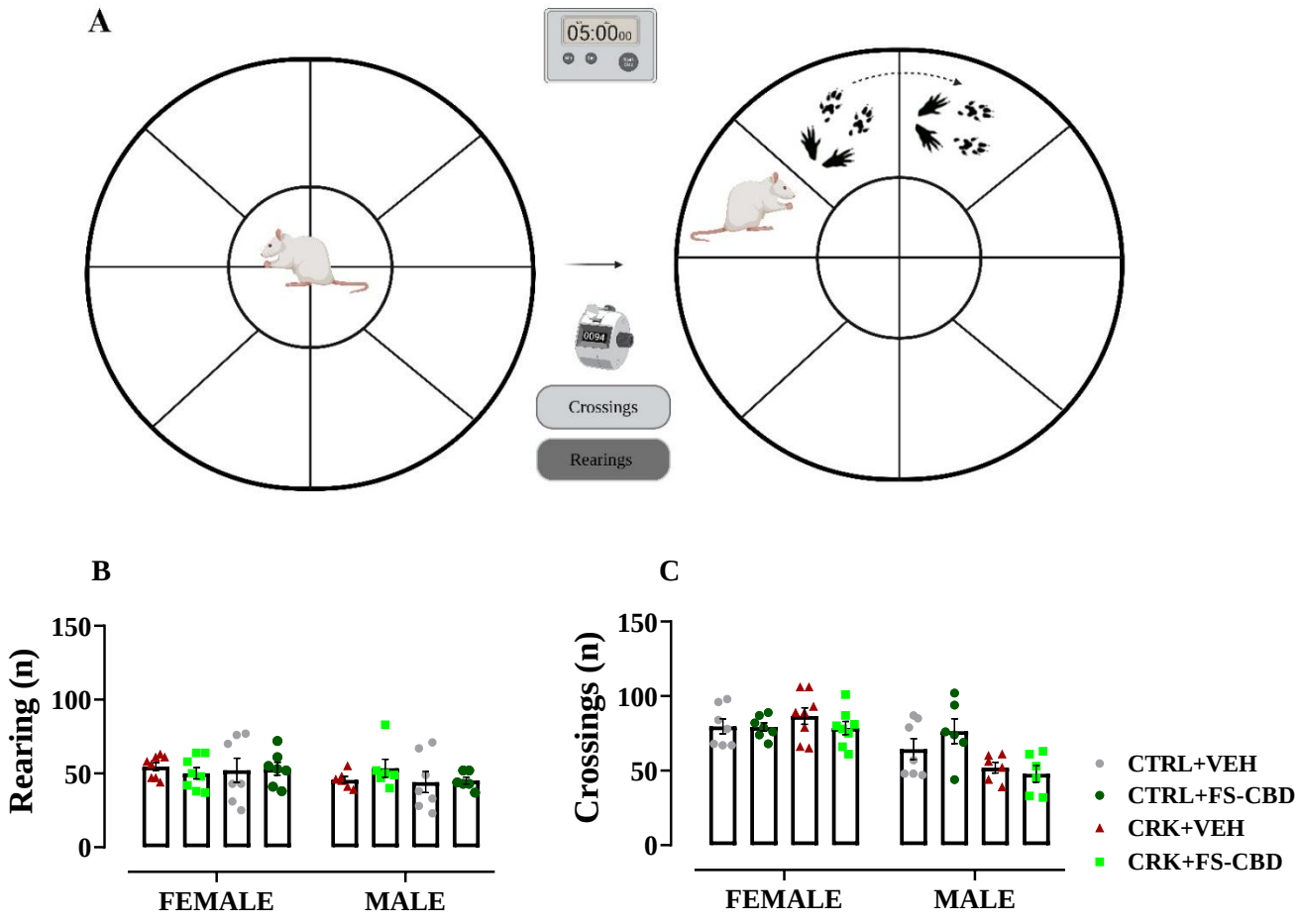

Figure 4. Effects of PN-Crack exposure and FS-CBD treatment on spontaneous locomotor activity assessed in

the open field test (OF Schematic representation of the OF test methodology (A). No significant differences were observed between the studied groups for the number of rearings (B) and crossings (C). Data are expressed as mean ± SEM for 8-10 rats per group, analyzed by three-way ANOVA with Tukey's post-hoc test. CRK, crack; FS-CBD, full-spectrum *Cannabis sativa* oil rich in cannabidiol; CTRL, control; VEH, vehicle.

2.1 Effects of FS-CBD treatment on depressive-like behavior following PN-Crack exposure

To assess whether PN-Crack exposure induces depressive-like behaviors in F1 animals, we used the Forced Swim Test (FST) **(Figure 5A)**. Animals exposed to PN-Crack showed a significant reduction in the latency to immobility compared to the control group (CRK+VEH vs CTRL+VEH) in both males (p=0.05) and females (p=0.02), suggesting depressive-like behavior **(Figure 5B)**. FS-CBD treatment was not able to block this behavior.

Regarding the total time of immobility, we found that animals exposed to PN-Crack exhibited a significant increase in immobility time compared to the control group (CTRL+VEH vs CRK+VEH) (p=0.01 for females; p=0.03 for males), reinforcing depressive-like behavior. However, a promising result was observed in the males treated with FS-CBD, who showed a significant reduction in immobility time compared to animals exposed to PN-Crack (CRK+VEH vs CRK+FS-CBD; p=0.02 for males) **(Figure 5C)**. PN-Crack exposure induced depressive-like behaviors in both males and females, and FS-CBD treatment was able to reverse one of these parameters in a sex-dependent manner, showing an effect only in males.

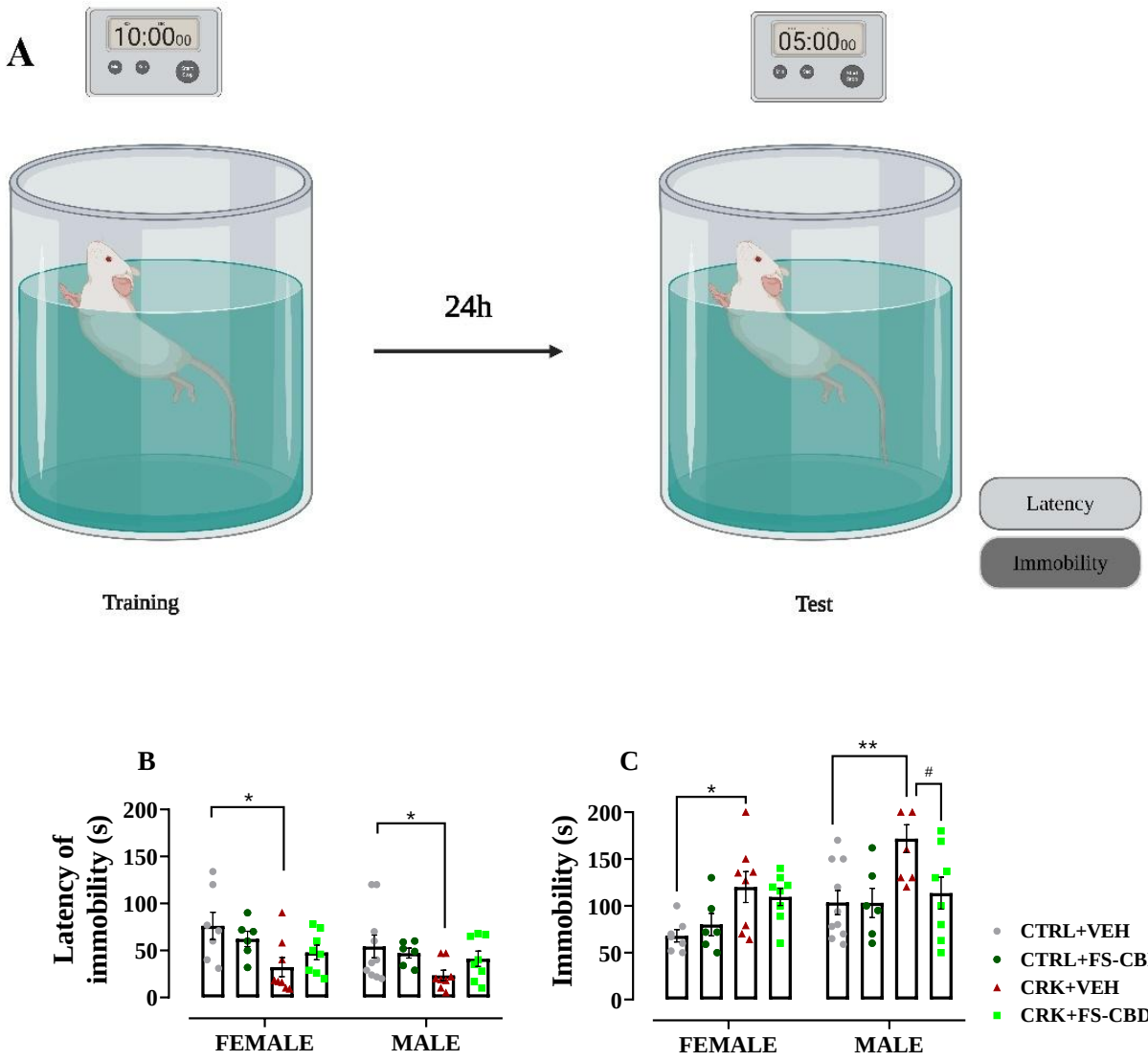

Figure 5. Effects of PN-Crack exposure and FS-CBD treatment on behavior assessed in the Forced Swim Test (FST). Schematic of the FST methodology (A). Males and females exposed to PN-Crack showed a shorter latency to immobility (4 minutes) compared to the control group (B). Males and females exposed to PN-Crack exhibited a longer immobility time compared to control animals during the FST, and FS-CBD reduced immobility time compared to PN-Crack animals (C). Data represent the mean ± SEM of 8-10 rats per group. *p<0.05 compared to the CTRL+VEH group, #p<0.05 compared to the CRK+VEH group. ANOVA three-way with Tukey's post-hoc test. CRK, crack; FS-CBD, full-spectrum *Cannabis sativa* oil rich in cannabidiol; CTRL, control; VEH, vehicle.

2.2 Effects of PN-Crack exposure and FS-CBD treatment on long-term memory

To evaluate the effects of PN-Crack exposure and FS-CBD treatment on long-term memory, we used the Object Recognition Test (ORT) **(Figure 6A)**. The three-way ANOVA analysis revealed no significant differences in the DI (Discrimination Index) after exposure to crack and/or FS-CBD treatment, with all evaluated groups presenting p>0.05 **(Figure 6B)**.

Similarly, regarding the percentage of PI (Preference Index), the results also showed no significant differences between groups. Both males and females exposed to PN-Crack exhibited exploration times similar to those of the control group, regardless of FS-CBD treatment (p>0,05) **(Figure 6C)**. PN-Crack exposure did not induce long-term memory impairments, preventing the observation of potential therapeutic effects of FS-CBD on this parameter.

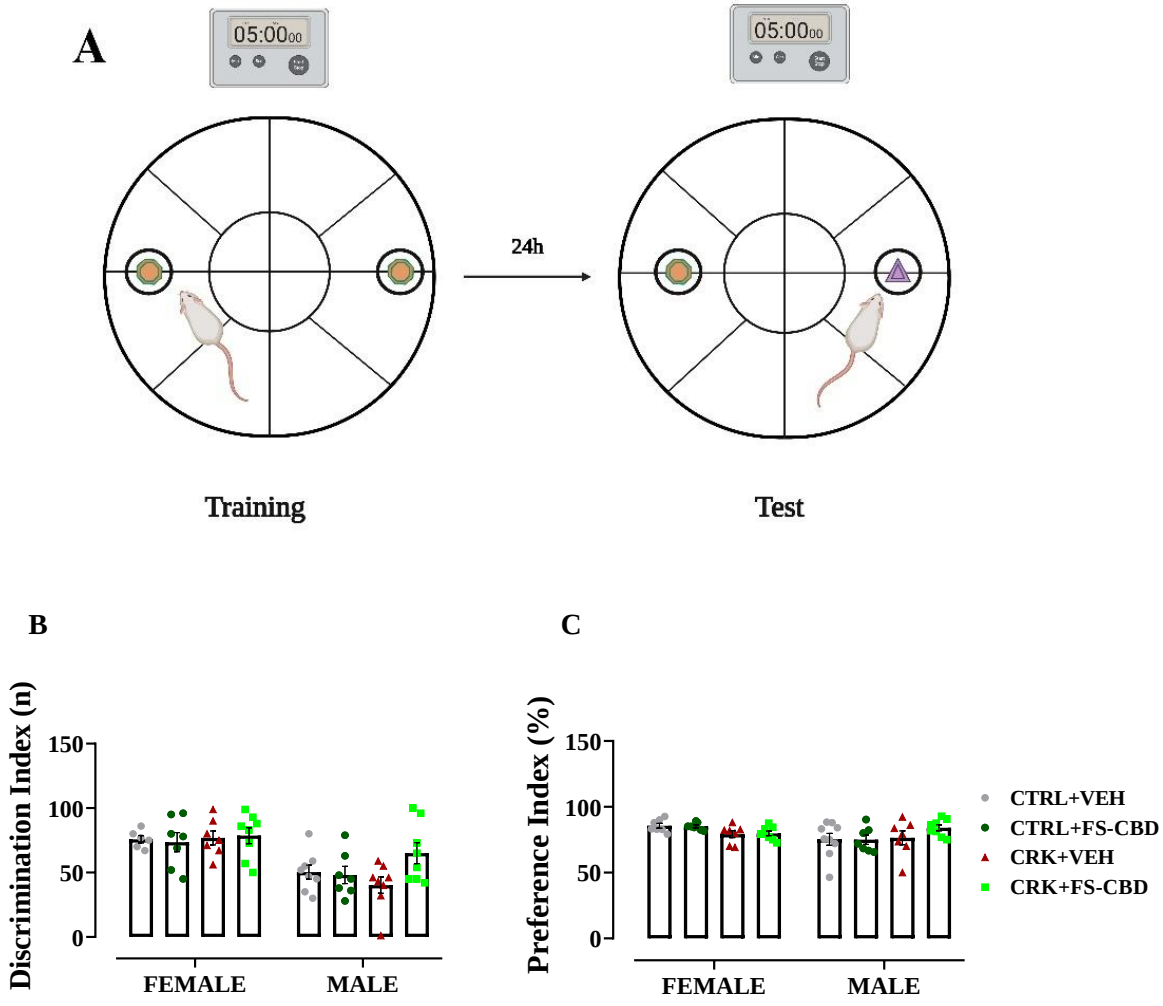

Figure 6. Effects of PN-Crack exposure and FS-CBD treatment on long-term memory consolidation in young animals. Schematic of the methodology for the Object Recognition Test (ORT) (A). No significant differences were observed between the studied groups for the Discrimination Index (DI) (B). No significant differences were found for the Preference Index (PI) (C). The data represent the mean ± SEM of 8-10 rats in each group. Three-way ANOVA with Tukey's post-hoc test. CRK, crack; FS-CBD, full spectrum *Cannabis sativa* oil rich in cannabidiol; CTRL, control; VEH, vehicle.

2.3 Effects of PN-Crack exposure and FS-CBD treatment on immune system organs

Evaluating the effects of PN-Crack exposure and FS-CBD treatment on immune system organs (**Figure 7A**), the three-way ANOVA analysis did not identify significant differences in the number of thymocytes and splenocytes between the experimental groups

(p>0.05). Animals exposed to PN-Crack from both sexes showed similar cell counts to those observed in the control group. Additionally, FS-CBD treatment did not show any relevant effect on these immune parameters (**Figures 7B and C**).

Regarding the relative weight of the thymus and spleen, no significant differences were observed between the groups for thymus weight in both males and females, with all groups showing p>0.05. Additionally, FS-CBD treatment did not have a significant effect on the weight of any of the organs analyzed (p>0.05) (**Figures 7D**). However, females exposed to PN-Crack exhibited a trend toward reduced spleen weight compared to the control group (CTRL+VEH vs CRK+VEH; p=0.06, females; **Figures 7E**). PN-Crack exposure had no significant impact on immune parameters, except for a trend toward reduced spleen weight in females, which limited the potential therapeutic effects of FS-CBD.

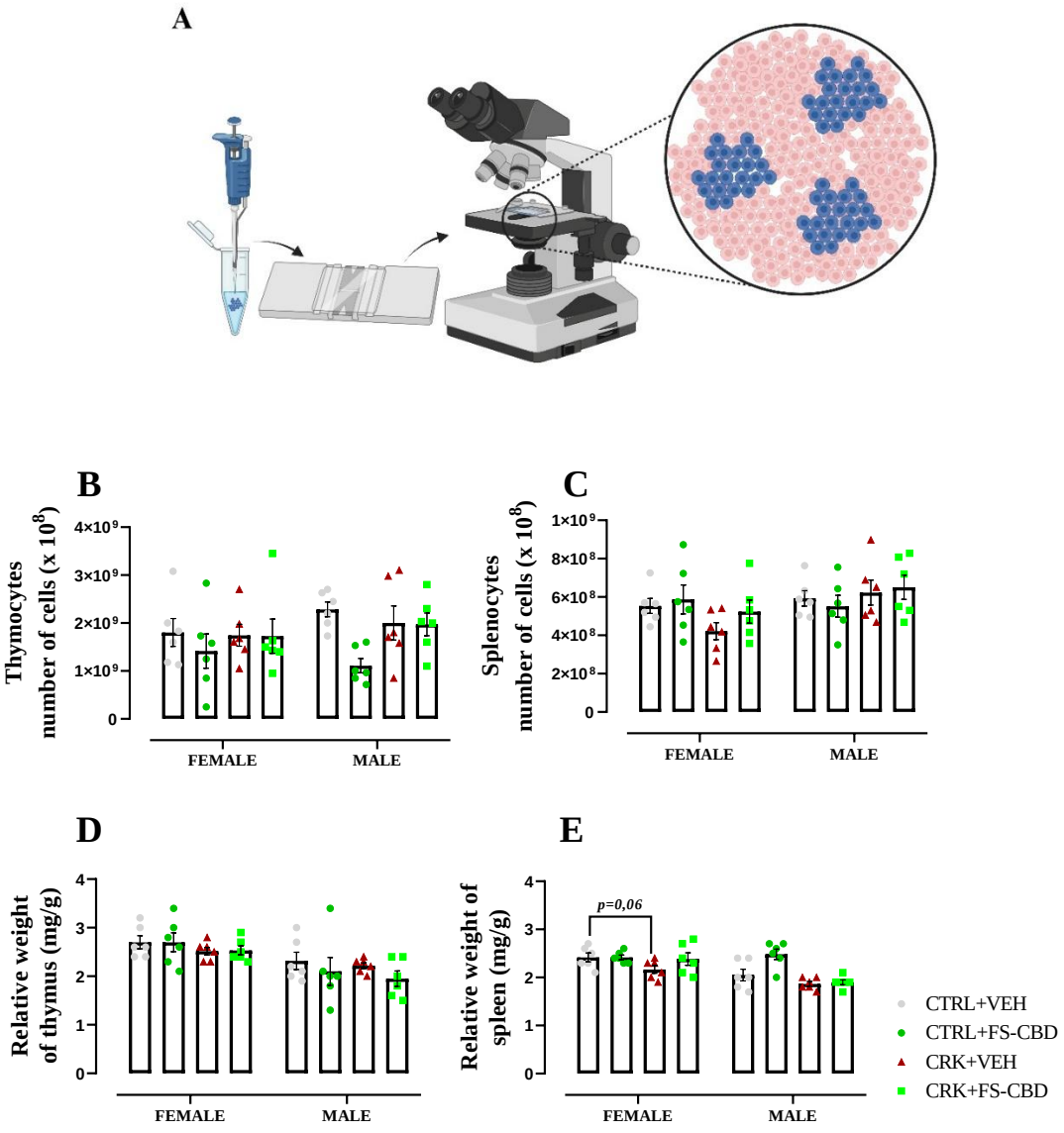

Figure 7. Effects of PN-Crack exposure and FS-CBD treatment on immune parameters and organ relative weights. Schematic representation of the methodology for thymus and spleen cellularity analysis (A). No significant differences were observed between the groups for total thymocyte count (B), total splenocyte count (C), or relative thymus weight (D). A trend toward reduced relative spleen weight was observed in animals exposed to PN-Crack compared to the control group (p=0.06) (E). Data are expressed as mean ± SEM for 8-10 rats per group. Analysis performed by three-way ANOVA with Tukey's post-hoc test. CRK, crack; FS-CBD, full-spectrum C. sativa oil rich in cannabidiol; CTRL, control; VEH, vehicle.

2.4 Effects of PN-Crack exposure and FS-CBD treatment on salivary glands and salivary flow

The analysis of salivation parameters (**Figure 8A**) revealed that the saliva volume was significantly reduced in males exposed to PN-Crack compared to the control group (CRK+VEH vs CTRL+VEH; males; p=0.0150; Figure 8B). Regarding salivary flow, a significant decrease was observed in males exposed to PN-Crack compared to the control group (CTRL+VEH vs CRK+VEH; males; p=0.0295; **Figure 8B**), while females exposed to PN-Crack exhibited a significant increase in this parameter compared to the control group (CTRL+VEH vs CRK+VEH; p=0.0333; **Figure 8C**). However, FS-CBD treatment did not induce significant differences in salivary volume or flow in either sex.

Regarding the weight of the salivary glands, no significant differences were found for the weight of the left or right submandibular glands in both sexes in the analyzed groups (**Figures 8D and 8E**; p> 0,05**)**. Regarding the right parotid gland, a significant increase in weight was observed in males treated with FS-CBD and exposed to PN-Crack compared to males exposed only to PN-Crack (CRK+FS-CBD vs CRK+VEH; p=0.0038). In contrast, in females, a significant reduction in the weight of the right parotid gland was observed in animals exposed to PN-Crack compared to the control group (CTRL+VEH vs CRK+VEH; females; p=0.0096; **Figure 8F**). Finally, analysis of the left parotid gland weight revealed a significant decrease in both sexes in animals exposed to PN-Crack compared to the control group (CTRL+VEH vs CRK+VEH; females; p=0.0499; males; p=0.0209) **(Figure 8G)**. These results highlight the distinct effects of PN-Crack exposure and FS-CBD treatment on salivary function and the structural characteristics of the salivary glands, with variations depending on sex and the type of gland evaluated.

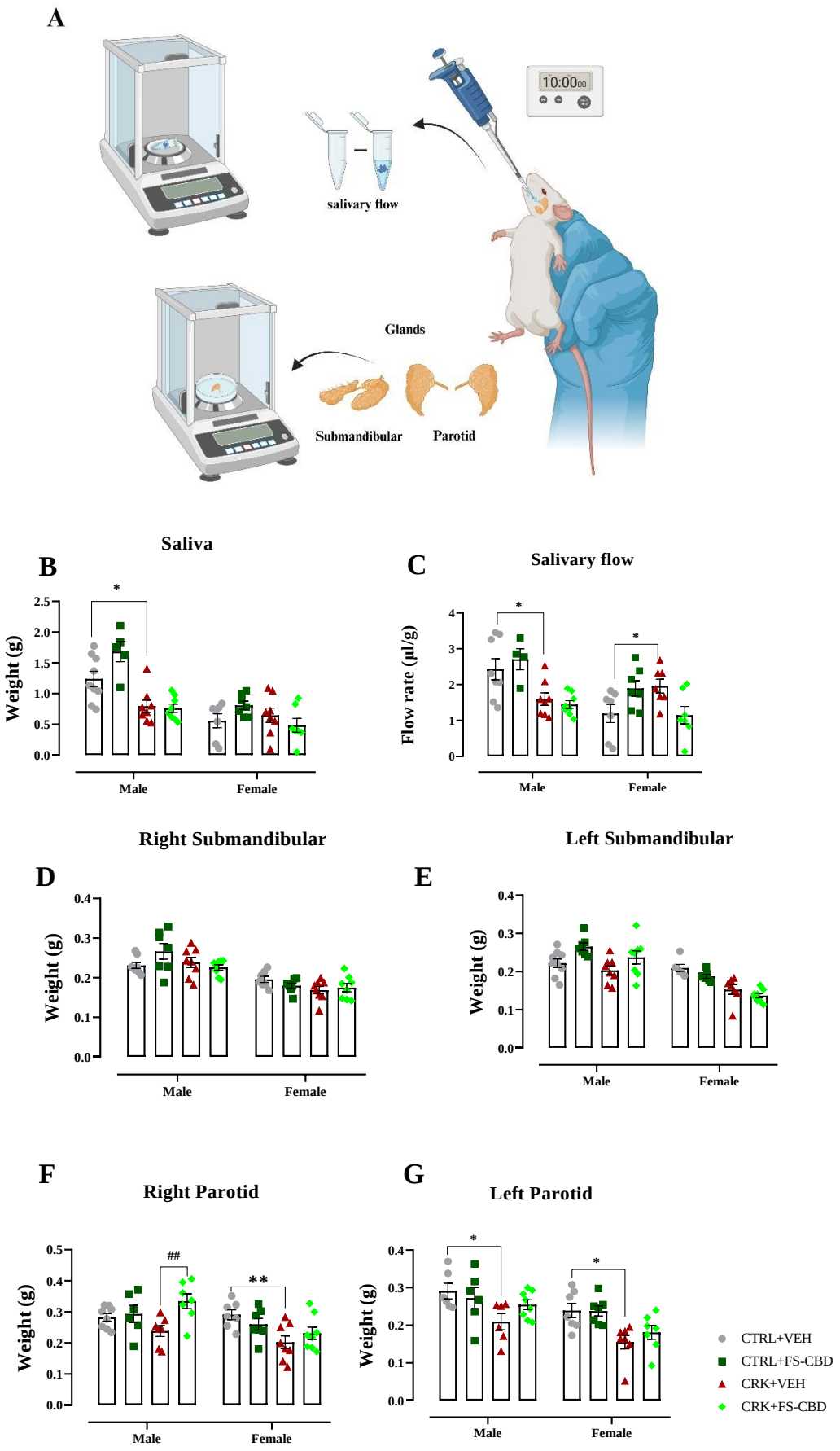
A
10:00
salivary flow
Glands
Submandibular
Parotid
Saliva
B
Weight (g)
Male
Female
Salivary flow
C
Flow rate (µl/g)
Right Submandibular
D
Left Submandibular
E
Right Parotid
F
Left Parotid
G
CTRL+VEH
CTRL+FS-CBD
CRK+VEH
CRK+FS-CBD

Figure 8. Scheme of methodology for salivary glands and salivary flow (A). Significant reduction in salivary volume in males exposed to PN-Crack (B). Salivary flow showed decreases in females and increases in males exposed to PN-Crack (D-F). No significant differences were observed in the weight of submandibular glands (E). Right parotid glands exhibited an increase in females treated with FS-CBD and exposed to PN-Crack compared to females exposed to PN-Crack, and a decrease in males exposed to PN-Crack (G). Left parotid glands showed a reduction in both sexes exposed to PN-Crack. Data represent the mean ± SEM of 8-10 rats per group. Three-way ANOVA with Tukey's post-hoc test. CRK, crack; FS-CBD, full-spectrum C. sativa oil rich in cannabidiol; CTRL, control; VEH, vehicle.

4. DISCUSSION

This study revealed for the first time that PN-Crack exposure induced lasting sex-dependent changes in behavioral, physiological, and glandular alterations in adult F1 rats. The reduction in weight gain observed in females exposed to PN-Crack and treated with FS-CBD reflects metabolic and hormonal changes caused by both substances, while males showed greater resistance to these changes. In terms of behavior, PN-Crack promoted anxiogenic and depressogenic-like effects, with FS-CBD partially reversing these effects, with responses dependent on sex. Despite not exhibiting significant cognitive deficits in long-term memory, the exposed animals exhibit indications of compensatory mechanisms that mitigate the impacts of PN-Crack. Furthermore, crack cocaine affected salivary function in a sex-specific manner, altering salivary flow and glandular weight. At the same time, FS-CBD showed a protective effect in one gland, also in a sex-differentiated manner.

The reduction in weight gain observed in females exposed to PN-Crack and those treated with FS-CBD suggests a physiological response to both substances. It is known that crack cocaine exposure during pregnancy can impair weight gain, with repercussions extending throughout postnatal development (Pacheco *et al.*, 2021). This effect can be explained by the action of cocaine and its metabolites, which cross the placental barrier and directly affect fetal development, altering metabolic and hormonal processes (Queiroz *et al.*, 2021). Exposure to crack cocaine can also make animals more vulnerable to fluctuations in body weight when exposed to other substances, such as CBD (Román-Vargas *et al.*, 2023).

Although CBD is widely recognized for its therapeutic properties, its effects on weight gain or loss remain inconclusive. Research indicates that these effects vary depending on the dose, duration of treatment, sex, and experimental conditions (McPartland *et al.*, 2015). However, the full-spectrum oil used in this study includes THC, which may also influence the animals' weight. THC is known to modulate appetite and metabolism, and it can interact with CBD in complex ways, impacting body weight differently depending on the hormonal and metabolic profile of the animals (Eitan *et al.*, 2023). The differential response between males and females suggests that sexual factors play a crucial role in

modulating the effects of both substances. Furthermore, the interaction between the endocannabinoid system and sex hormones, such as estrogen may explain these differences, as estrogen modulates cannabinoid receptors, affecting the response to THC and CBD (Tabatadze *et al.*, 2015).

Estrogen has been shown to influence the expression and sensitivity of CB1 and CB2 receptors, as well as the levels of endocannabinoids like anandamide, impacting synaptic plasticity, mood regulation, and energy homeostasis (Maia *et al.*, 2017a). This modulation occurs através do aumento da expressão dos receptores CB1 e CB2 em determinadas regiões do cérebro, bem como da inibição da enzima FAAH (fatty acid amide hydrolase), responsável pela degradação da anandamida, resultando em uma maior disponibilidade desse endocanabinoide (Maia *et al.*, 2017b). This mechanism may make females more susceptible to changes in body weight because of these compounds, as the combination of the hormonal cycle with cannabinoids may enhance the regulation of appetite and metabolism differently between sexes (Eitan *et al.*, 2023). Therefore, further studies are needed to investigate these differences more thoroughly, considering the complexity of the interactions between sex hormones and the endocannabinoid system.

This study stands out for evaluating animals in adulthood, which allowed us to identify how exposure to PN-Crack continues to modulate anxious-like behavior at more advanced stages of life. Assessing different life stages reveals the persistence and evolution of crack cocaine neurobehavioral impacts, suggesting that these effects may manifest more complexly with development (Oliveira; Bosch, 2023). Regarding anxious-like behavior, exposure to PN-Crack led to a significant reduction in the %TBA of the EPM, characterizing an anxiogenic-like behavior. These results are consistent with our previous studies, which also investigated the effects of crack cocaine exposure in experimental rodent models (Pacheco *et al.*, 2021), demonstrating that the substance leads to long-lasting and persistent alterations in the behavior of animals that need to be better studied.

The FS-CBD treatment showed a tendency to increase the %TBA in a sex-dependent manner. This result is consistent with studies that demonstrate the anxiolytic effects of CBD, with strong evidence of its effectiveness in stress and anxiety animal models (Austrich-Olivares *et al.*, 2022). The differential response observed between males and females reflects the influence of sex hormones on the endocannabinoid system. Estrogen modulates CB1 and CB2 receptors, which may increase the sensitivity of females to the anxiolytic effects of FS-CBD (Walker; Holloway; Raha, 2019). There is compelling evidence suggesting that 17β-estradiol can induce the overexpression of CB1 and CB2 receptors, with CB2 also playing a

role in anti-inflammatory processes. Given that CBD interacts with both CB1 and CB2 receptors, it is likely that its effects through these pathways are interconnected rather than independent. Therefore, these findings are essential for better understanding the variability of the response to FS-CBD between the sexes and the underlying mechanisms.

Exposure to PN-Crack also reduced the latency to immobility and increased the total time spent immobile in the FST, which is indicative of depressive-like behavior. These findings corroborate the literature, which points to the depressogenic-like effects of crack cocaine in animal models, such as the comorbidity between anxiety and depression disorders (Hurt *et al.*, 2009). The use of abused substances, such as crack cocaine, can affect the hypothalamic-pituitary-adrenal (HPA) axis function, leading to alterations in cortisol levels and other stress-related hormones, which in turn may contribute to depressive behaviors (Sampedro-Piquero *et al.*, 2020). Although treatment with FS-CBD did not produce significant effects in females, it was observed that males exposed to crack cocaine and treated with FS-CBD showed a significant reduction in immobility time, suggesting an antidepressant-like effect of FS-CBD.

This finding is consistent with studies highlighting the antidepressant effects of CBD, particularly in stress-induced models (Campos et al., 2013; Crippa et al., 2020; García-Gutiérrez et al., 2020; Shbiro et al., 2019). The antidepressant effect of CBD is often attributed to its ability to interact with the serotonergic and endocannabinoid systems since both are involved in regulating mood and emotional behavior (Crippa *et al.*, 2020). However, the lack of effect in females may be attributed to the interaction between sex hormones and the endocannabinoid system (Levine *et al.*, 2021). Hormonal analysis in future studies may clarify the physiological basis of these sexual differences in response to treatment.

Regarding cognitive deficits, research indicates that prenatal exposure to psychoactive substances, such as crack cocaine, can affect neurodevelopment and impair cognitive functions, especially memory and learning (Pacheco *et al.*, 2021; Singer *et al.*, 2005). However, these effects are not universal, varying according to the experimental protocol, the age of the animals, and the parameters evaluated. In this study, PN-Crack exposure did not result in significant cognitive deficits in long-term memory in animals at P60. The absence of significant differences in discrimination and preference indices may reflect compensatory mechanisms in the brains of animals exposed to crack cocaine. Regions such as the hippocampus may develop adaptations that mask behavioral deficits in specific tests, indicating the need for complementary assessments, such as analyses of oxidative

stress or neuroinflammation (Poladian *et al.*, 2023). Although the current results do not show significant differences, this does not invalidate the hypothesis that FS-CBD may attenuate memory deficits induced by substances such as crack cocaine, possibly through its neuroprotective and anti-inflammatory properties (Patricio *et al.*, 2020). Recent studies suggest that CBD may modulate endocannabinoid signaling in a way that restores neuroplasticity and reduces oxidative stress, promoting memory recovery in models of cognitive dysfunction (Hickey *et al.*, 2024).

Investigating the effects of prenatal exposure to PN-crack and FS-CBD treatment on lymphoid organs, specifically regarding thymocytes and splenocytes, the results of this study indicate that exposure to PN-Crack did not provoke significant changes in their counts, corroborating other studies that suggest that although the use of psychoactive substances like crack cocaine can affect immune function, cellular effects are not always evident in terms of cell count (Bagasra; Forman, 1989; Baldwin; Roth; Tashkin, 1998; Ruiz *et al.*, 1994). A study by Butler; Rehm; Fischer, (2017) observed a decrease in thymic T-cell function in individuals exposed to crack cocaine, but with no direct changes in cell count. This phenomenon may be related to underlying mechanisms, such as a change in the functional quality of the cells rather than a reduction in the absolute number of cells (Ruiz *et al.*, 1994). Furthermore, it is crucial to consider that prenatal exposure to crack cocaine may have differential effects compared to direct exposure to the substance, with potential long-term consequences such as alterations in immune response and cellular maturation, which may not be apparent in cell counts.

The analysis of the relative weight of the spleen showed a trend toward a reduction in spleen weight in females exposed to PN-crack, although it did not reach statistical significance ($p=0.06$). This finding may align with studies that observe that chronic use of substances like crack cocaine can lead to changes in the morphology and weight of lymphoid organs (Kessler et al. 2008). Chronic cocaine use induces the release of norepinephrine and prevents its reuptake by adrenergic nerve endings, leading to an accumulation of neurotransmitters, thus causing constriction of the spleen (Oliveira; Neves; Farias, 2021).

Treatment with FS-CBD did not show significant effects on the count of thymocytes and splenocytes nor on the relative weight of the thymus and spleen. However, it is essential to highlight that these results may be related to the fact that exposure to PN-Crack did not cause significant negative effects on the evaluated immunological parameters, except for a trend toward reduced relative spleen weight in females. This suggests that, in the absence of substantial changes induced by crack cocaine, there was no significant immunological

stressor for FS-CBD treatment to act upon. In general, the effects of CBD on the immune system may be modulated by factors such as concentration and experimental context. In animal models of inflammation, CBD has demonstrated the ability to attenuate the migration and infiltration of inflammatory cells (Booz, 2011). According to Martini et al., (2023) although CBD may exert modulatory effects on the inflammatory response and immune cell activity, its effects on immune cell counts are not always evident in all experimental settings. Furthermore, the direct immunomodulatory effects of CBD on immune system organs, such as the thymus and spleen, are still underexplored in the literature, highlighting the need for further investigations to understand its therapeutic potential in this context better.

Discussing the results regarding the effects of PN-Crack exposure and FS-CBD treatment on salivary glands and salivary flow reveals unprecedented and highly relevant scientific data. The reduced saliva volume and flow in males exposed to PN-Crack indicates a potential glandular dysfunction. These findings are consistent with previous studies that point to crack cocaine's influence on the exocrine system, although such studies typically address direct substance use rather than prenatal exposure (Antoniazzi *et al.*, 2017).

On the other hand, the increased salivary flow observed in females exposed to PN-Crack suggests sexual differences in the response mechanisms to the substance, possibly modulated by hormonal factors or differences in the development of the salivary glands. Literature on sexual variations in the impact of drugs on glandular functions is limited, but a study conducted with other psychoactive substances suggests that estrogen and testosterone may influence glandular and metabolic responses to drug effects (Kim *et al.*, 2020). In contrast, (Santana-Melo *et al.*, 2025) also reported that direct exposure to crack for 14 consecutive days in males leads to an increase in salivary flow when stimulated by pilocarpine, which reinforces the idea of sex-dependent differences and the type of exposure, whether direct or prenatal, in the glandular responses to the drug.

The absence of significant changes in the weight of the submandibular glands, in contrast to the changes observed in the parotid glands, highlights that different types of salivary glands may respond differently to prenatal crack cocaine exposure. This may reflect variations in vascularization, innervation, or the glands' sensitivity to chemical substances (Kim *et al.*, 2020).

The positive impact of FS-CBD on the increased weight of the right parotid gland in males exposed to PN-Crack suggests a possible protective effect. This is in line with evidence that CBD can modulate inflammatory and oxidative processes, as well as promote tissue regeneration (Hickey *et al.*, 2024). On the other hand, the reduction in the weight of

the right parotid gland in females and the left parotid gland in both sexes reinforces findings indicating that the parotid gland is more responsive to exogenous variations and contributes less to resting salivary flow when not stimulated by chewing or taste (Carpenter, 2013). These results emphasize the need for further studies to elucidate the underlying mechanisms, considering the sexual differences in glandular functions.

In summary, this study revealed new insights into the long-lasting effects of prenatal crack cocaine exposure, showing that even in more advanced life stages, in an animal model, prenatal crack cocaine exposure affects behavior and salivary function. Additionally, the findings regarding FS-CBD treatment suggest that, although FS-CBD has promising effects, the sex-dependent and the need for adjustments in dose or treatment duration must be considered to maximize its therapeutic benefits.

5. CONCLUSION

This study has a significant impact on the lives of children born to crack cocaine users, as it provides valuable insights into the long-term effects of prenatal crack exposure, particularly regarding behavior, physiology, and glandular function, with variations observed between sexes. The finding that FS-CBD treatment showed promising results, particularly in males, opens up the possibility of offering an innovative therapeutic alternative to reduce the adverse effects of crack exposure, such as depressive behaviors and glandular dysfunction. Furthermore, the findings could contribute to the development of more effective and personalized treatments that take sexual differences into account, potentially improving the quality of life for these individuals by offering intervention options to mitigate long-term damage and promote well-being. This may lead to new pathways for the treatment of neuropsychiatric disorders and other complications associated with prenatal crack exposure, thereby enabling a more tailored therapeutic approach to the specific needs of these individuals.

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ANEXO 1. CEUA APROVADO

UNIVERSIDADE FEDERAL DE ALAGOAS
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que a proposta intitulada *"Efeitos do canabidiol sobre as alterações neuropsicofarmacológicas em mães e filhotes expostos ao crack durante período gestacional"*, registrada com o **nº 28/2021**, sob a responsabilidade do pesquisador **Prof. Dr. Olagide Wagner de Castro**, que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), **para fins de pesquisa científica,** encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela Comissão de Ética no Uso de Animais da Universidade Federal de Alagoas (CEUA/UFAL), em reunião de 30 de junho de 2022.

Vigência da autorização	16.08.2022 a 16.08.2026
Espécie/linhagem/raça	Rato Wistar
Nº de animais	200
Peso/idade	150g – 300 g / 60 – 90 dias
Sexo	Machos e fêmeas
Origem / Local de manutenção	Biotério Central da Ufal / LNFI - ICBS
Colaboradores	Jucilene Santos, Amanda Pacheco, Igor Melo, Maisa Costa, Yngrid Santos, Fernanda Souza, Bianca Silva, Keila Oliveira, Milenna Reiter, Kellyson Oliveira e Noemi Torres.

Maceió, 15 de agosto de 2022.

gov.br Documento assinado digitalmente
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