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RESEARCH ARTICLE

Neurobehavioral and molecular changes in rats exposed to either captagon or counterfeit captagon

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Abstract

The primary ingredient in captagon, a medication that is frequently abused in the Middle East, is fenethylline (FEN), which breaks down into theophylline and amphetamines (AMP). Due to the limited supply of genuine Captagon, fake Captagon (CC) has surfaced, comprising a variety of chemicals such as lidocaine, theophylline, AMP, caffeine, and diphenhydramine. This study compares the neurobehavioral consequences of CC with FEN, emphasizing the higher health concerns associated with CC. A total of 36 male Sprague Dawley rats were split up into five groups: CC (50 or 100 mg/kg), FEN (50 or 100 mg/kg), and control. Following therapy, body temperature and locomotor activity were recorded. Using quantitative real-time PCR (qRT-PCR), the expression of the Brain-Derived Neurotrophic Factor (BDNF) gene was examined in prefrontal cortex (PFC) samples. The findings indicated a higher risk of lethal hyperthermia because CC significantly increased body temperature and locomotor activity in comparison to FEN. Furthermore, a significant reduction in BDNF mRNA levels in the PFC following CC exposure raised the possibility of long-term cognitive and neuroplasticity deficits. According to these results, CC poses a significantly bigger risk because of its unexpected composition and more severe neurobehavioral effects, even though FEN is a recognized social menace. The present study underscores the pressing necessity of public health measures to curb the proliferation and misuse of CC. To lessen these new medications' detrimental effects on people and society as a whole, education about their risks and initiatives to stop their usage are crucial.

Keywords Addiction · Fenethylline · Captagon · BDNF · Behavioral study

1 Introduction

Addiction is a chronic relapsing disorder characterized by the loss of inhibitory control over drug use and frequent relapses despite negative consequences (Maldonado et al. 2021). It is widely recognized that drug addiction is a serious public health crisis that affects almost every aspect of a community in some manner, ultimately resulting in social disharmony, felony offenses, degraded social standing, and even physical and mental health issues (Rasel et al. 2018). Importantly, smoking, alcohol, and illicit drug abuse are responsible for 11.8 million deaths worldwide annually. According to the Global Burden of Disease study, this number is greater than deaths resulting from cancer and represents one-fifth of all deaths reported globally (Cheron and Kerchove d'Exaerde 2021).

Captagon (FEN) is a central nervous system stimulant that was initially developed in the 1960s for the treatment of depression, narcolepsy, and attention deficit hyperactivity disorder (Kristen et al. 1986; Nickel et al. 1986; Nevešćanin et al. 2008). The active element in captagon is fenethylline, a recognized psychoactive compound, which is chemically structured as amphetamine connected to theophylline via an alkyl chain. Fenethylline contains two primary active metabolites in humans: amphetamine and theophylline (Musshoff 2000; Van Hout and Wells 2016). Amphetamine is a well-known stimulant that primarily mediates its effects by increasing norepinephrine and dopamine (DA) levels in the body (Keup 1986; Koob et al. 2014). Amphetamine abusers seek euphoria, increased energy, and better concentration afforded by the drug (Koob et al. 2014). Notably, amphetamine use in large doses can cause severe psychiatric symptoms such as hallucinations and paranoia, which could potentially

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induce acute psychosis (similar to paranoid schizophrenia) (Koob et al. 2014).

FEN was one of the most widely abused drugs in the Middle East (Katselou et al. 2016), banned and withdrawn in 1988, which, in turn, resulted in increased illicit production ((DEA 2003). According to the United Nations and the National Committee for Narcotics Control and Anti-Drug and Preventative Affairs, this drug accounted for 40% of drug abusers in Saudi Arabia (Katselou et al. 2016). The lack of sophisticated instrumentation, as well as cheap and legal raw materials required for synthesis, contributed to its production in clandestine laboratories and abuse in the Middle East (Katselou et al. 2016). Fenethylline's unique composition, linking amphetamine and theophylline by an alkyl chain, sets it apart from other stimulants. Despite its therapeutic origins, Captagon (FEN) became widely abused, particularly in the Middle East, leading to its ban and the emergence of counterfeit versions.

Based on analytical research that assessed 124 batches of captagon tablets, fenethylline was not detected in examined captagon tablets, whereas (theophylline, amphetamine, caffeine, diphenhydramine, and lidocaine) were found in counterfeited ones (DEA 2003). Counterfeit captagon (CC), in this context, refers to drugs marketed as captagon but lack the active ingredient, fenethylline. They include other substances, creating a dangerous mix with unpredictable effects. These CC tablets may meet drug abuse requirements for the abusers but threaten their health (Alabdalla 2005).

CC comprises several ingredients, their precise combined effects remain unknown on health. In general, psychostimulants are known for their recreational use and long-term effects on brain chemistry and function. Moreover, these substances have insidious ramifications on various physiological functions, with one of the notable effects being increases in locomotor activities and the provocation of hyperthermia. Recently, their impact on the brain derived neurotrophic factor (BDNF) expression has gained substantial interest due to its implications on neuroplasticity, cognitive function, and mental health.

The objective of this study was to compare, in rats, the effects of CC and FEN on locomotor activity, body temperature and the mRNA expression of BDNF in the Prefrontal cortex (PFC), as this could provide better insights into their potential effects on brain function. To the best of our knowledge, no study has reported the neurobehavioral changes in rats exposed to either FEN or CC.

2 Materials and methods

2.1 Animals

Thirty-six male Sprague Dawley rats, aged 8 weeks, were divided into five groups: control (vehicle), FEN50 (50 mg/kg), CC50 (50 mg/kg), FEN100 (100 mg/kg), and CC100 (100 mg/kg). Rats were provided by the Saudi Food and Drug Authority (SFDA; Riyadh, Saudi Arabia). Animals were kept in conventional plastic tubes with controlled humidity (30%) and temperature (21 °C) on a 12:12 light–dark photoperiod for 7 days before being tested. Throughout the experiment, rats were given unlimited food and water. During the light cycle, all experiments were carried out. The experimental techniques of the animal study were authorized by Taif University's Research Ethics Committee in compliance with the National Institutes of Health's Institutional Animal Care and Use Committee requirements. Body temperature and locomotor activity were recorded at baseline and every 30 min post-injection. PFC samples were analyzed for BDNF mRNA expression using qRT-PCR.

2.2 Drugs

FEN and CC were donation from the General Directorate of Narcotics Control in Saudi Arabia. Saline solution (0.9% NaCl) was used to reconstitute all drugs used in this study.

2.3 Experimental design

Experimental design was illustrated in Fig. 1. Rats were randomly divided into five groups as follow: 1. The first group is the control (Ctrl) group that received a single intraperitoneal (i.p.) injection from the vehicle (1 mL/kg, i.p.); 2. FEN50 group received a single low dose of the FEN (50 mg/kg, i.p.); 3. CC50 group received a single low dose of the CC (50 mg/kg, i.p.); 4. FEN100 group received a single high dose of the FEN (100 mg/kg, i.p.); 5. CC100 group received a single high dose of the CC (100 mg/kg, i.p.). After 30 min of the dose, animals were assessed in an open field test to investigate the locomotor changes. The body temperature was recorded during baseline and every 30 min following the injection to yield 10 readings in order to investigate the changes in body temperature following exposure to these drugs. After 24 h of the injection, animals were euthanized by life decapitation. Half of the brains were immediately frozen and stored in −80-degree Celsius (Fig. 1).

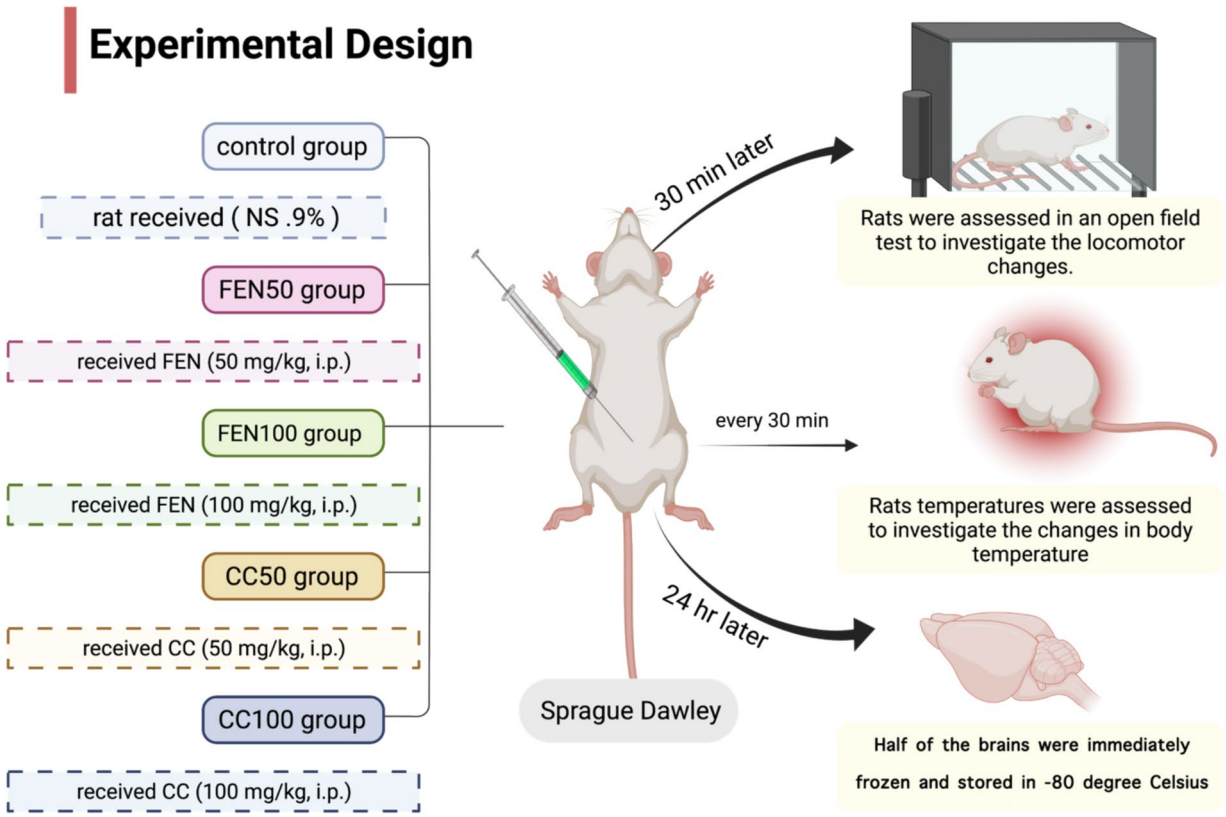

Fig. 1 Experimental setup of locomotor and thermoregulatory assessments in Sprague Dawley rats. The study includes five groups: (1) Control Group: 6 Rats received normal saline solution (NS 0.9%) intraperitoneally (i.p.). (2) FEN50 Group: 6 Rats were administered FEN (50 mg/kg, i.p.). (3) FEN100 Group: 9 Rats were administered FEN (100 mg/kg, i.p.). (4) CC50 Group: 6 Rats were administered CC (50 mg/kg, i.p.). (5) CC100 Group: 9 Rats were administered CC (100 mg/kg, i.p.). After treatment, rats underwent behavioral assessments in an open field test to evaluate locomotor activity. Their body temperature was also recorded to observe any thermoregulatory changes. Post-experiment, half of the rat brains were harvested, frozen, and stored at −80 °C for future biochemical analyses

2.4 Locomotor activity

Any possible effects of FEN and CC rat's motor activity were investigated by assessing distance travelled 30 min following the injection. Motor activity was assessed over three minutes by quantifying total distance traveled, using an ANY- maze video tracking system.

2.5 Body temperature

Body temperature was recorded during baseline and every 30 min following the injection to yield ten readings in order to investigate the changes in body temperature following exposure to these drugs.

2.6 Brain tissue harvesting

Brains were obtained from freshly sacrificed male Sprague Dawley rats. The brains were quickly removed and immediately covered in dry ice and stored at −80 °C until cryosectioning. Brains were cryosectioned at 15 to 40 µm thickness. PFC was identified using stereotaxic according to the Rat Brain Atlas and extracted manually using a scalpel from the brain. These brain regions were collected on Eppendorf tube and were immediately stored at −80 °C.

2.7 Total RNA isolation

Twenty five samples from 5 different male Sprague Dawley rats groups were used in this study, RNA samples from the brain tissue groups were obtained from the PFC. Total RNA was extracted using the Trizol (Ambion), According to previously published study and as demonstrated previously (Stone et al. 2007). The RNA isolation protocol consists of the following steps:1- The cells were resuspended in 1 ml trizol; 2- Then, 200 µl of chloroform was added to each sample; 3- The samples were mixed and incubated at room temperature for 5 min;4- The samples were centrifuged for 15 min at 12000 rpm at 4° C; 5- The top aqueous layer was transferred to a new tube with 500 µl isopropanol; 6- The samples were mixed gently and incubated at room temperature for 10 min; 7- The samples were centrifuged

for 15 min at 15000 rpm at 4° C to pellet the RNA; 8- The supernatant was removed and the samples were washed with 500 µl of 75% ethanol by spinning for 5 min at 13000 rpm at 4° C;9- The ethanol was aspirated from all the samples and the pellets were left to dry for 5 min at room temperature; 10—The pellets were resuspended in 50 µl RNAse free water; 11—The samples were mixed for 10 min at 56° C on heatblock; 12—RNA concentration was measured using MaestroNano (2 µl / sample).

2.8 RNA quality control

The quality of the isolated RNA was determined using Maestro Nano Micro-Volume Spectrophotometer (MAESTROGEN). This instrument was used to detect accurate concentration in addition to 260 nm, 280 nm, 230 nm absorbance ratios. The sample reader was cleaned with RNase free water and dried with a kim wipe. 2 µl of the RNase free water was used as blank, and then 2 µl of each isolated RNA sample was loaded and the readings were recorded. The sample reader was cleaned thoroughly between samples.

2.9 Quantitative real-time PCR (qRT-PCR) analysis

RT-PCR analysis was performed using RNA samples were isolated from PFC of rats in each group according to (W.M. Keck Foundation Biotechnology Microarray Resource Laboratory at Yale (Freitas et al. 2021). BDNF mRNA expression in various samples were quantified by using GoTaq® 1-Step RT-qPCR System kit (Promega Corporation), followed by an amplification reaction using the CFX384 Real-Time system (BIO-RAD, USA) with corresponding primers obtained from Promega Corporation (Madison, USA) according to the manufacturer's instructions. BDNF mRNA levels were normalized to GAPDH, was used housekeeping gene for normalization of our raw data and compared with a reference sample. The fold change was based on the △△C real-time PCR method with 2 sample repetitions for each group. The PCR program for relative quantification of lncRNA was as follow: first, denaturation at 95 °C for 10 min; followed by 45 cycles of denaturation for 15 s at 95 °C; then annealing for 30 s at 55 °C and extension for 30 s at 70 °C.

2.10 Statistical analysis

The data obtained were statistically analyzed using Graph Pad PRISM-9 Software and expressed as mean ± standard error of the mean (SEM). The locomotor activity data and data of BDNF mRNA expression were evaluated using One-way ANOVA Followed by Tukey's multiple comparison test. Body temperature data was analyzed using two-way repeated measure ANOVA (Treatment X Time). In all comparisons, differences were considered significant at $P < 0.05$.

3 Results

3.1 Locomotor activity

One – way ANOVA test revealed a significant difference in locomotor activity between groups ($F = 22.85$, $P < 0.0001$) as shown in Fig. 2. The Tukey's multiple comparisons test showed that, there was a significant increase in CC50 group compared to Ctrl group ($P = 0.0392$) and in CC100 group compared to Ctrl group ($P < 0.0001$) (Fig. 2). A significant increase in distance travelled was found in CC100 as compared to CC50 group ($P < 0.0009$). No significant changes were detected in distance travelled between Ctrl and FEN50 groups ($P > 0.9999$) or between Ctrl and FEN100 groups ($P = 0.9652$). There were also no significant changes in distance travelled between FEN50 and FEN100 groups ($P = 0.9642$). On the other hand, there was a significant increase in CC50 group compared to FEN50

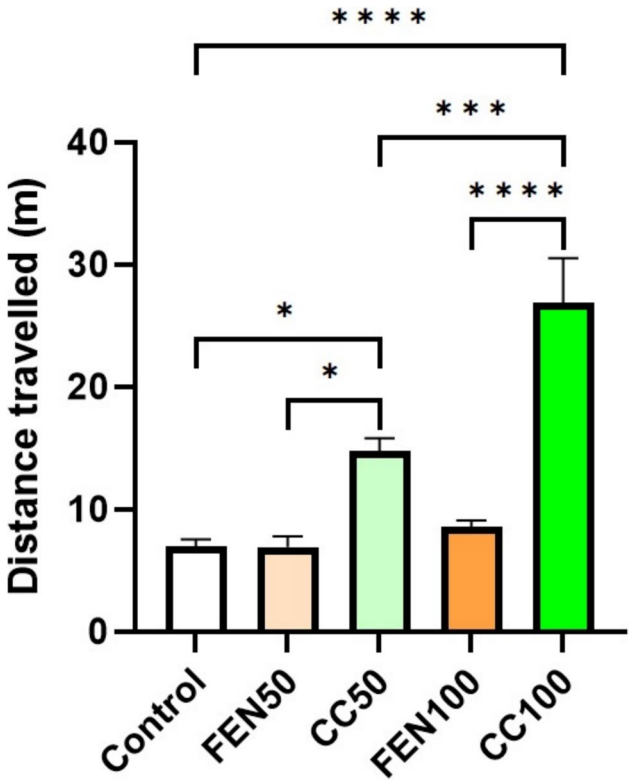

Fig. 2 Distance travelled in meters for three minutes period after injections by 30 min in the five groups of rats: (Control) group received a single intraperitoneal (i.p.) injection of the vehicle (1 mL/kg, i.p.); (CC50) group received a single low dose of the Counterfeit Captagon (50 mg/kg, i.p.); (CC100) group received a single high dose of the Counterfeit Captagon (100 mg/kg, i.p.); (FEN50) group received a single low dose of Fenethylline (50 mg/kg, i.p.); (FEN100) group received a single high dose of Fenethylline (100 mg/kg, i.p.).Values are shown as means ± standard errors of the means

($P = 0.0387$) and in CC100 group compared to FEN100 group ($P < 0.0001$).

3.2 Body temperature

The changes of body temperature have been analysed using two-way repeated measure ANOVA (Treatment X Time). The analysis revealed a significant main effect of time ($F(10, 200) = 69.78$, $P < 0.0001$), a significant effect of treatment ($F(4, 20) = 154.3$, $P < 0.0001$) and a significant Treatment × time interaction ($F(40, 200) = 18.84$, $P < 0.0001$; Fig. 3).

Tukey's multiple comparisons test showed that the temperature in the Ctrl and FEN50 groups remained unchanged during all test points.

In the CC50 group, the temperature was significantly increased ($P < 0.0001$) as compared to the Ctrl group and FEN50 group approximately in the timepoint 90 min until timepoint 270 minutes after CC50 injection, whereas the values of CC50 group were not changed significantly as compared to the Ctrl group and FEN50 group at 300 min till the completion of the study.

In the FEN100 group, the temperature was increased ($P < 0.0001$) as compared to the Ctrl group and FEN50 group in the timepoint 210 min and 240 min respectively after FEN 100 injection. While the temperature of FEN100 group were not significantly changed as compared to FEN50 in the timepoint 90 min until 270 min as well as not significant among the Ctrl group and FEN50 group at 270 min till the completion of the study. A significant increase was demonstrated in the CC100 temperature value ($P < 0.0001$) started from the timepoint 90 min until 330 min compared to the Ctrl group, FEN50 group, CC50 group and FEN100 group.

3.3 Changes in BDNF mRNA levels in PFC

An RT-qPCR technique was performed to assess the changes in BDNF mRNA Levels that were affected by low doses (50 mg/kg, i.p.) and high doses (100 mg/kg, i.p.) of FEN and CC in the PFC region of the brain. To test the changes in gene expression, we used one way ANOVA test which revealed a significant main effect in the relative expression of mRNA of the BDNF enzyme ($F = 19.58$, $P < 0.0001$).

The Tukey's multiple comparisons tests showed that the relative expression of mRNA of the BDNF enzyme was not significantly changed in the FEN100 group as compared to the Ctrl group ($p = 0.0823$). Yet, the relative BDNF mRNA levels were significantly decreased in the CC100 group as compared to the Ctrl group ($p < 0.0001$). Moreover, the expression was not significantly changed in the FEN50 group as compared to the FEN100 group ($p = 0.6359$) and in the CC50 groups as compared to the CC100 group ($p = 0.2239$) (Fig. 4). On other hand, the expression was significantly decreased in the CC50 group as compared to the FEN50 group ($p = 0.0039$). There was also a significant decrease in the CC100 group as compared to the FEN100 group ($p = 0.0006$).

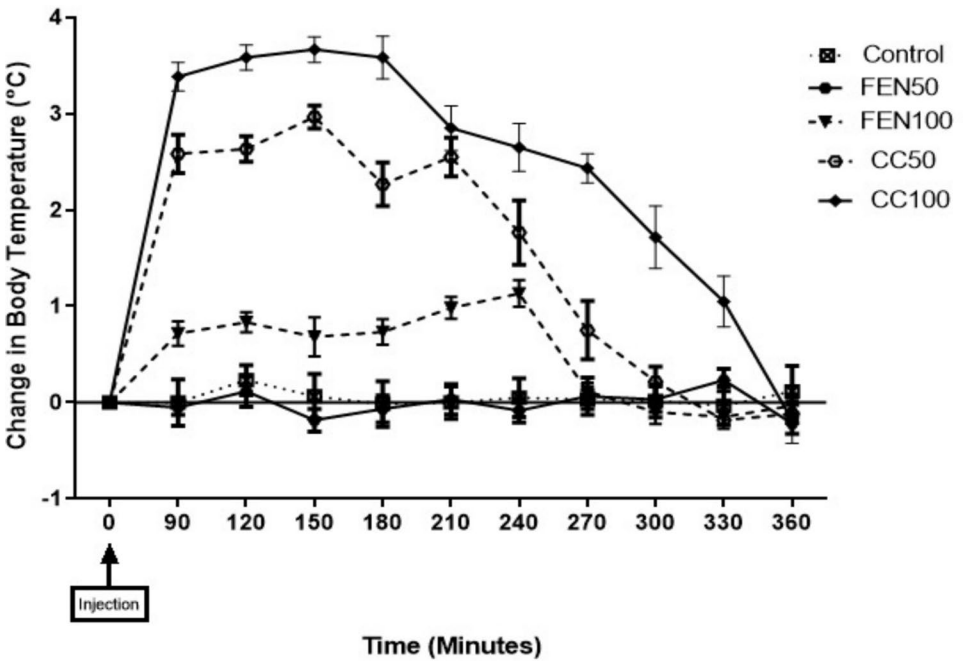

Fig. 3 The changes of body temperature were affected by two different types of Captagon, FEN with low and high doses (FEN50 and FEN100), and CC with low and high doses (CC50 and CC100). The timepoint starts at 90 min to 360 min. Values are shown as means ± standard errors of the means. Stars were removed for clarity

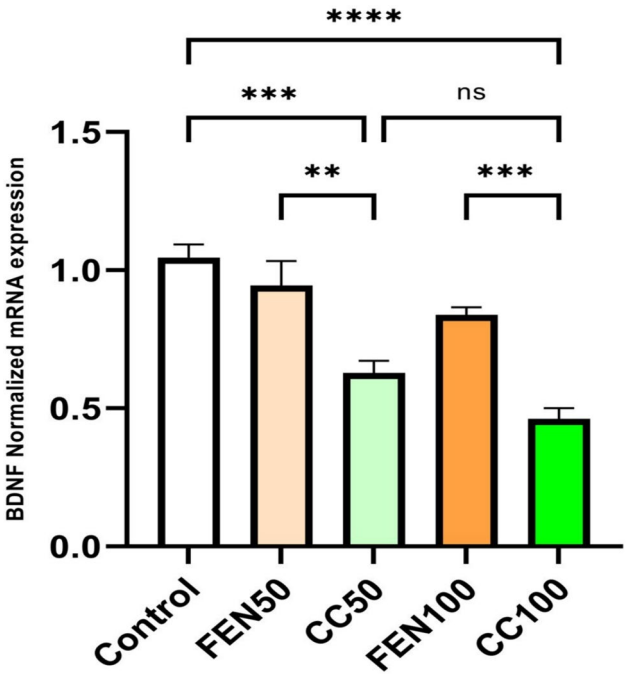

Fig. 4 Change in BDNF mRNA level in PFC in the five groups of rats: (Control) group received a single intraperitoneal (i.p.) injection of the vehicle (1 mL/kg, i.p.); (CC50) group received a single low dose of the Counterfeit Captagon (50 mg/kg, i.p.); (CC100) group received a single high dose of the Counterfeit Captagon (100 mg/kg, i.p.); (FEN50) group received a single low dose of Fenethylline (50 mg/kg, i.p.); (FEN100) group received a single high dose of Fenethylline (100 mg/kg, i.p.).Values are shown as means ± standard errors of the means

4 Discussion

Captagon abuse becomes a dangerous threat because it can be addicted faster and cause serious health problems (Fong et al. 2024; Shalaby et al. 2023). Young users prefer CC since it is easy to get and costs less than original FEN. The present study has demonstrated that increasing CC dose also increased locomotor activity in the tested rats. There was a significant movement increase in CC groups compared to FEN groups, which led us to ensure the risk of CC. These findings are consistent with previously studies of stereotyped behavior and locomotor activity following acute, chronic, and continuous amphetamine administration (Nielsen 1981; Robinson and Becker 1986; Schiørring 1979). The amphetamine -induced locomotion activity based on the effects of this drug on the dopaminergic neurotransmission (Di Lullo SL and Martin-Iverson MT 1991). The neurochemical and behavioral effects are substrates of the monoamine transporters and the vesicular monoamine transporter causing the release of monoamines, disrupting the reuptake process. That will increase extracellular 5-HT, DA and noradrenalin activity in the brain (Müller et al. 2007; Schuldiner et al. 1993). The effects include (hyperthermia, increased intracellular and extracellular DA, glutamate induced excitotoxicity, oxidative stress, microglia activation, and apoptotic pathway activation). The presence of multiple active substances in CC can result in complex drug interactions, leading to different pharmacological outcomes. For example, caffeine and amphetamine are known stimulants that can increase body temperature and locomotor activity, while paracetamol is an antipyretic that may reduce body temperature. Such interactions can obscure the specific contributions of individual ingredients to the observed effects. To mitigate these issues, future studies should aim to standardize the composition of CC samples used for experimental comparisons (Alhazmi et al. 2020; Alshehri et al. 2020; Katselou et al. 2016). Creating a standardized formulation for CC will provide more precise and dependable comparisons with FEN. Moreover, comprehensive chemical analysis of each batch of CC must be performed to ascertain the precise composition and address possible variances.

The observed rise in locomotor activity in rats administered CC, especially at elevated doses, highlights the compound's strong stimulatory effects, likely due to its amphetamine-like characteristics. This aligns with prior research indicating heightened locomotor activity subsequent to the administration of amphetamine derivatives, recognized for their capacity to augment dopaminergic and noradrenergic neurotransmission within the central nervous system (CNS) (Schiørring 1979). The lack of a comparable rise in activity with FEN, particularly at the lower dosage, indicates divergent effects of these drugs on the central nervous system (CNS), either attributable to differences in their pharmacokinetic profiles or unique modes of action.

More recent evidence suggests that decreased levels of 5-HT transporters and 5-HT content in the rat hippocampus are result of prolonged administration of 3,4-methylenedioxymethamphetamine (MDMA) (Rebec et al. 1997). On the other hand, the current study was to characterize the relative potency of FEN and CC in comparing the severity of damage in terms of the changes in the rat's body temperature. Previous studies showed that hyperthermia, a prominent and critical clinical response to stimulants intoxication, was also listed as the cause of microglial activation. Which causes damage to DA nerve endings, while the other neurochemical effects resulting from methamphetamine (METH) encourage overflow of DA into the synapse, thus high body temperature causes death if the body temperature exceeds 41.3 (Thomas et al. 2004). This may explain the sharp increases of body temperature with high dose of CC100 as compared to CC50, FEN100 and FEN50. Interestingly, the body temperature findings reveal a complex interplay between the doses of FEN and CC and their thermogenic effects. The significant rise in body temperature in the CC50 and CC100 groups, compared to the control, could reflect the enhanced metabolic and muscle activity associated with increased

locomotor behavior, as well as direct thermogenic effects of these substances on the hypothalamic centers regulating body temperature. The differential response in body temperature changes between the FEN and CC groups highlights the unique pharmacological properties of CC, which may include more pronounced effects on the body's thermoregulatory mechanisms.

The CC contains caffeine which have suppressive effect on TPH expression in rats (Lim et al. 2001). Paracetamol is an ingredient in CC as well that causes hypothermia in humans and rodents (Coman et al. 2022). The main ingredients in CC are AMP, theophylline, paracetamol, and caffeine are related to body temperature changes. AMP, theophylline, and Caffeine can raise the body temperature while Paracetamol minimize it. FEN stimulates the central nervous system (CNS), enhancing alertness, concentration ability, and physical performance, while providing a sense of well-being and appetite suppression. In low to moderate doses, it produces bronchial vessel dilation and increases in heart rate, body temperature, respiration, and blood pressure (Katselou et al. 2016). That explains the greatest damage happened by CC, which raises the body temperature and locomotor activity more than FEN whether in high and low doses.

According to Kiyatkin and Sharma (2011), METH exposure causes changes in histochemical and morphological parameters. Heat shock protein (HSP 72kD) expression was examined after METH intoxication and showed a massive and wide changes in neural and glial cells that checked deeply in the cortex, hippocampus, thalamus, and hypothalamus which is correlated with brain temperature elevation. The main factor of neurotoxicity as well is METH exposure, that leads to oxidative stress, high temperature, and oedema (Kiyatkin and Sharma 2011).

Interestingly, in this study, different doses of CC modified the BDNF mRNA levels significantly as compared to the FEN in PFC region of the brain. There was a significant decrease in BDNF mRNA expression in the CC100 group compared to that in the FEN100 group. Repeated abuse of the stimulant drugs such as AMP is associated with extra neuronal DA accumulation in specific brain areas. DA oxidative metabolism generates ROS, namely Hydrogen peroxide (H_2O_2) (Fleckenstein et al. 1997; Cunha-Oliveira et al. 2006). Prior research has shown that AMP correlates with alterations in antioxidant enzymes, suggesting that oxidative stress has a role in the long-term effects of these addictive pharmaceuticals (Cunha-Oliveira et al. 2006).

BDNF, a neurotrophic factor extensively distributed in the brain, is crucial for the survival and functionality of dopaminergic neurons and for safeguarding them against harmful influences (Ren et al. 2016; He et al. 2020). In addition to its critical role in neuronal survival and growth, it serves as a neurotransmitter modulator and contributes to neuronal plasticity, which is crucial for memory and learning (Scalzo et al. 2020; Huang and Reichardt 2001). The changes in BDNF mRNA levels in the PFC following the injection of CC and FEN are very significant. BDNF is essential for the survival, growth, and maintenance of neurons, and it is implicated in learning, memory, and advanced cognitive abilities. The marked reduction in BDNF mRNA levels in the CC100 group compared to the control indicates that elevated dosages of CC may hinder neuroplasticity and perhaps lead to cognitive impairments. This discovery corresponds with findings from human research and animal models suggesting that prolonged exposure to amphetamines may result in neurotoxic consequences and cognitive deficits. In the experiment, we observed a reduction in the mRNA expression of the neurotrophic factor BDNF in the PFC region following the administration of CC (50 – 100 mg/kg). The study's findings align with other research indicating a decrease in BDNF levels in the PFC region due to AMP use (Chaves Filho et al. 2020; Valvassori et al. 2019).

The lack of substantial alterations in BDNF mRNA levels in the FEN100 group, relative to the control, may suggest a diminished adverse effect of FEN on neuronal health and plasticity at the administered doses. However, the similar decrease in BDNF expression in the CC50 group relative to FEN50 suggests that even at lower doses, CC may have more pronounced effects on neuroplasticity than FEN. This could be due to the presence of other active compounds in CC that were not investigated in this study, which could synergistically contribute to its neuropharmacological effects.

Previously, AMP led to time-dependent increased in BDNF expression mRNA levels within mice's PFC, hippocampus, and amygdala (Fries et al. 2015). In relation to PFC, there is some disagreement in the literature; some studies have reported a decrease in BDNF in the PFC following AMP treatment (Valvassori et al. 2019). However, some have reported increased neurotrophins levels (Shen et al. 2014), while others have reported no change (Stertz et al. 2013). It is known that BDNF is one of the most abundant neurotrophies in the brain and that it plays an important role in the survival of cholinergic and dopaminergic neurons (Angelucci et al. 2007; Conner et al. 1998). Further, declines in the production of Neurotrophins, as well as deficits in their utilization, participate in the pathogenesis of depression and schizophrenia (Angelucci et al. 2004; Shoval and Weizman 2005).

Also, it's becoming clear that oxidative stress may play an instrumental role in ensuring neurotoxic effects (Sayre et al. 2008; Kuloglu et al. 2002; Ozcan et al. 2004; Ranjekar et al. 2003). Although overwhelming data indicates that oxidative stress significantly contributes to AMP-induced neurotoxicity (Cadet and Brannock 1998; Brown and Yamamoto 2003),

there is a lack of knowledge regarding the effects of AMP on antioxidant enzymes (Jayanthi et al. 1998). Our study examined the neurobehavioral effects of counterfeit Captagon (CC) vs fenethylline (FEN) in male Sprague Dawley rats. We noted substantial elevations in locomotor activity and body temperature in the CC-treated animals, accompanied by a reduction in BDNF mRNA levels in the prefrontal cortex (PFC). These findings offer new insights into the potential health hazards associated with CC, while also underscoring the complexity of result interpretation due to the varied makeup of CC.

5 Limitations and future directions

This study offers important insights into the neurobehavioral effects of CC and FEN, though it is essential to recognize certain limitations. A significant limitation of the study is the acute exposure paradigm employed, which may inadequately reflect the long-term neuropharmacological effects of CC and FEN. Chronic administration protocols used in psychostimulant research may produce varying outcomes, especially concerning neuroadaptive changes and modifications in BDNF expression. The heterogeneous and unpredictable composition of CC poses challenges for experimental standardization and reproducibility. The variability in active substances among different batches of CC hinders direct comparisons with other psychostimulants and restricts the generalizability of our findings.

A further limitation is that our findings regarding BDNF levels are based exclusively on mRNA data acquired through qRT-PCR. This method is useful for gene expression analysis; however, it does not yield direct measurements of BDNF protein levels, which are essential for comprehending the functional implications of BDNF regulation. Future research should integrate quantitative protein analysis techniques, including Enzyme-Linked Immunosorbent Assay (ELISA) and Western Blot (WB), to enhance mRNA data and deliver a more thorough evaluation of BDNF expression.

Future studies should prioritize the standardization of CC sample formulations utilized in experimental comparisons. Conducting detailed chemical analyses of each batch will account for potential variations in composition and enhance the accuracy and reliability of results. Furthermore, examining the long-term effects of chronic CC exposure on neurobehavioral outcomes and neuroplasticity will provide a more thorough understanding of the neuropharmacological impact of these substances. Investigating the interactions among the active substances in CC and their collective effects on the CNS is essential for clarifying the distinct pharmacological properties of CC in relation to other psychostimulants.

Additionally, undertaking region-specific research, especially in urban areas of Saudi Arabia, will yield significant insights into the local dynamics of substance abuse. Such studies can facilitate the collection of extensive data on drug abuse patterns, socio-economic effects, and particular obstacles encountered in various places. Ultimately, targeted public health interventions and educational initiatives must be enhanced to increase knowledge regarding the hazards of CC, especially among at-risk populations. Policies designed to regulate the production and distribution of counterfeit medications must be strengthened to alleviate their detrimental effects on public health.

6 Conclusions

The present investigation shown that brain tissues were markedly impacted by the toxicity caused by CC. Notably, CC, in contrast to FEN, resulted in greater elevations in body temperature, hence increasing the risks of fatal hyperthermia. Furthermore, this CC resulted in a substantial enhancement of locomotor activity relative to FEN. The observed behavioral changes were associated with substantial reductions in PFC mRNA levels of BDNF in the CC group. These findings represent the preliminary evidence indicating that CC may pose greater health risks than FEN. Increased measures are necessary to restrict the utilization of these novel medications and to inform our society about their hazards. Our research provides essential insights into the immediate effects of FEN and CC on body temperature, locomotor activity, and BDNF mRNA expression in rats, along with the ramifications for individuals and society. These results highlight the intricate connections between these medications and the CNS, with possible consequences for their misuse and the emergence of cognitive and neurophysiological deficits. Additional research is necessary to investigate these findings more thoroughly and to evaluate the long-term effects of exposure to these drugs.

Although this work offers significant insights into the immediate effects of CC and FEN on locomotor activity, body temperature, and BDNF mRNA expression, we recognize the constraints imposed by the diverse characteristics of CC. Standardizing the composition of CC in future study will be essential for clarifying the distinct neuropharmacological mechanisms and potential health hazards linked to these chemicals. Mitigating these limitations will improve the validity and generalizability of the findings, hence facilitating the development of more effective public health initiatives to address counterfeit drug usage.

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Declarations

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