



Research Article

Opioid-Induced Dysregulation of BDNF Signalling: A Study of Acute, Sub-Chronic, and Chronic Exposure in a Rodent Model

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Abstract

Brain-derived neurotrophic factor (BDNF) is a critical regulator of neuronal survival, synaptic plasticity, and cognitive function, making it a key biomarker in understanding the neurobiological impact of opioid exposure. This study investigated the effects of opioids (codeine and tramadol) on brain-derived neurotrophic factor (BDNF) levels in male Wistar rats. A total of 75 male Wistar rats (120–140g) were used for the study in acute, sub-chronic and chronic phases, using 25 rats for each phase. In each phase, 25 male Wistar rats were divided into 5 groups of 5 animals each. Group 1 served as the control and received distilled water, groups 2 and 3 were treated with tramadol at 20 and 60 mg/kg, respectively, while groups 4 and 5 were supplemented with codeine at 10 and 30 mg/kg, respectively. Treatment by oral gavage lasted for 14, 28 and 56 days for the acute, sub-chronic and chronic studies, respectively. Serum and hippocampal BDNF assays were analysed following standard laboratory protocols. Results showed dose and time-dependent significant reductions ($p < 0.05$) in BDNF levels across all treatment groups compared to controls, with the most pronounced decreases observed in codeine 30mg/kg and tramadol 60mg/kg groups. These findings suggest that prolonged opioid exposure disrupts neurotrophic signalling, potentially contributing to neurodegenerative conditions.

Keywords

Brain-derived Neurotrophic Factor (BDNF), Opioids, Neurodegenerative Conditions

1. Introduction

The brain is a complex organ where billions of interconnected neurons form intricate networks, governing cognition, emotion, and behaviour. These neural circuits are supported by glial cells such as astrocytes and microglia, which are essential for maintaining homeostasis, regulating synaptic

transmission, and providing neuroprotection [1, 2]. Synaptic plasticity, the ability of synapses to strengthen or weaken over time, is fundamental to learning and memory. Neurotrophic factors, neurotransmitters, and receptor signalling maintain these connections [1, 3, 4]. Disruptions in these connections

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can impair neural communication and cause neurological and psychiatric disorders. Brain-derived neurotrophic factor (BDNF) is a key protein that supports neuronal survival, differentiation, and synaptic plasticity. It is widely expressed in the hippocampus, cortex, and other brain regions critical for memory and cognition. BDNF binds to Tropomyosin receptor kinase B (TrkB), activating intracellular signalling cascades that promote neurogenesis and synaptic remodelling [5, 6].

Opioids like codeine and tramadol are potent analgesics whose primary mechanism is the activation of μ -opioid receptors. These receptors are widely distributed in the brain, spinal cord, and peripheral organs, particularly in neural circuits that process pain, emotion, and reward. Activation of these pathways not only provides analgesia but also stimulates reinforcement mechanisms, promoting drug-seeking behaviour and the desire to repeat the experience [7-9]. Moreso, exposure to psychoactive substances such as opioids can disrupt BDNF signalling, which is linked to neurotoxicity, reduced BDNF expression, and impaired cognitive function [10, 11].

While opioid-induced neurotoxicity is increasingly recognised, the mechanisms through which opioids alter BDNF levels remain unclear, especially regarding the influence of dose and exposure duration. Existing research has focused largely on morphine and heroin [11-14], creating a knowledge gap regarding commonly misused prescription opioids like tramadol and codeine. Furthermore, while clinical data indicate reduced serum BDNF in chronic opioid users [10, 15]. However, translating these clinical observations to a mechanistic understanding requires controlled experimental models detailing how opioids alter BDNF concentrations concurrently in the peripheral circulation (plasma) and in critical central regions like the hippocampus. The present study focuses on tramadol and codeine because they are among the most widely misused prescription opioids in Nigeria and other regions, particularly among young adults [16-18]. Tramadol and codeine are often perceived as “safer” alternatives, leading to their widespread misuse and the potential implications for neurodegenerative conditions [16-18]. The present study aims to characterise the dose and time-dependent effects of tramadol and codeine on BDNF expression, quantifying changes in both serum and hippocampal tissue of male Wistar rats across acute, sub-chronic, and chronic exposure rat models.

2. Materials and Methods

2.1. Chemicals and Assay Kits

The following opioids were used: codeine phosphate (DSP, Newtech Lifescience LLP, India) and tramadol hydrochloride (TRALAM, Hab Pharmaceuticals and Research Limited, India). Brain-derived neurotrophic factor (BDNF) levels were quantified using a commercial ELISA kit (Bioassay Technology Laboratory, BT LAB, China). Formal approval for the

procurement of these restricted substances was obtained from the National Drug Law Enforcement Agency (NDLEA) of Nigeria. Their acquisition, handling, administration, and disposal were performed in full compliance with all applicable NDLEA regulations.

2.2. Laboratory Animals

A total of seventy-five (75) male Wistar rats weighing between 120 and 140 g were sourced from the animal facility of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria. Animals were acclimatised for two weeks before experimentation. They were maintained on standard rat chow and water ad libitum, housed in groups of five per cage under controlled conditions: 30-50% humidity, a 12-hour light/dark cycle, and ambient temperature of 25 °C.

2.3. Study Design

A total of seventy-five (75) male Wistar rats were first divided into three (3) phases, comprising twenty-five (25) male Wistar rats: acute, sub-chronic and chronic studies, each lasting 14, 28 and 56 days, respectively. In each phase, twenty-five (25) male Wistar rats were randomly divided into five (5) groups of five (5) animals. Group 1 served as the control and received distilled water, groups 2 and 3 were treated with tramadol at 20 and 60 mg/kg, respectively, while groups 4 and 5 were supplemented with codeine at 10 and 30 mg/kg, respectively. Treatment by oral gavage lasted for fourteen (14), twenty-eight (28) and fifty-six (56) days for the acute, sub-chronic and chronic studies, respectively.

2.4. Blood and Tissue Sample Collection

After each phase, the animals were anaesthetised using intraperitoneal injection of Sodium pentobarbital at 50 mg/kg and 3ml of blood collected via cardiac puncture. Blood was centrifuged, and the supernatant serum was collected and stored at 4°C for laboratory assay. Also, the brain tissue was removed, and the hippocampus was dissected from each hemisphere on an ice-cold surface and weighed. For homogenization, a 50 mg tissue pool from both hippocampal hemispheres was placed in ice-cold normal saline (0.9% NaCl; 3 mL) and mechanically homogenised using a tissue grinder in an ice-water bath. The resulting homogenate was centrifuged at 3,000 rpm for 10 minutes at 4 °C, and the supernatant was collected and used for BDNF assay [19, 20].

2.5. BDNF Laboratory Assay

BDNF concentrations in serum and hippocampal homogenates were quantified using ELISA kits (BT LAB, China). The assay employed antigen-antibody binding reactions with horseradish peroxidase (HRP) colourimetric detection. Reagents were equilibrated to room temperature before use. Fifty

microliters of standard solution were added to designated wells. For sample wells, 40 μ L of sample, 10 μ L of anti-BDNF antibody, and 50 μ L of streptavidin-HRP were added. Plates were incubated at 37 °C for 60 minutes, washed five times, and then treated with 50 μ L of substrate solution B. After 10 minutes of incubation in the dark at 37 °C, reactions were terminated with 50 μ L of stop solution. Optical density (OD) was measured at 450 nm using a microplate reader within 10 minutes of adding the stop solution.

2.6. Statistical Analysis

Data were analysed using IBM SPSS Statistics v25.0. Results were expressed as mean $\pm$ SEM. Comparisons between

groups were performed using one-way ANOVA at a 95% confidence level, followed by post hoc analysis with the Least Significant Difference (LSD) test. Statistical significance was set at $p \leq 0.05$.

2.7. Ethical Consideration

The care and use of animals in this research adhered to internationally recognised ethical principles. Humane endpoints were observed, and every procedure was designed to minimise pain, distress, and discomfort [21, 22]. The study protocol was reviewed and approved by the University of Port Harcourt Research Ethics Committee UPH/CEREMAD/REC/MM99/029).

3. Results

Table 1. Changes in serum levels of Brain Derived Neurotrophic Factor (BDNF) following administration of some opioids in rats.

Groups	BDNF (ng/L) Levels		
	Acute phase (14 days)	Sub-chronic phase (28 days)	Chronic phase (56 days)
Group 1 control (distilled water)	54.84 $\pm$ 2.40	50.66 $\pm$ 1.95	39.93 $\pm$ 2.49
Group 2 (tramadol 20mg/kg)	40.42 $\pm$ 4.27 ^a	37.64 $\pm$ 0.81 ^a	27.95 $\pm$ 1.61 ^a
Group 3 (tramadol 60mg/kg)	36.29 $\pm$ 6.98 ^a	33.64 $\pm$ 0.08 ^a	24.05 $\pm$ 4.39 ^a
Group 4 (codeine 10mg/kg)	38.82 $\pm$ 2.19 ^a	31.52 $\pm$ 0.81 ^a	24.21 $\pm$ 0.42 ^a
Group 5 (codeine 30mg/kg)	30.90 $\pm$ 1.62 ^{a,c}	25.09 $\pm$ 4.31 ^{a,c}	19.47 $\pm$ 3.63 ^{a,c}

Values are presented as Mean $\pm$ SEM, n= 5.

^a Significant difference when compared to the mean value of group 1 ($p < 0.05$)

^b Significant difference when compared to the mean value of group 2 ($p < 0.05$)

^c Significant difference when compared to the mean value of group 3 ($p < 0.05$)

^d Significant difference when compared to the mean value of group 4 ($p < 0.05$)

Table 1 displays the result on the changes in serum levels of Brain Derived Neurotrophic Factor (BDNF) following administration of some opioids in rats. The outcome in the acute phase of the study indicated a general reduction in the BDNF levels in all test groups when compared to those of the control group, and these reductions were all significant ($p < 0.05$) when compared to those of the control group. The codeine treated groups followed by those treated with tramadol had the lowest levels of plasma BDNF amongst the test groups, and the reduction in group 5 (codeine 30mg/kg) was significant ($p < 0.05$) when compared to that of the tramadol

60mg/kg-treated group. The result of changes in serum BDNF level under the sub-chronic phase followed a similar trend to that in the acute phase. The outcome under the chronic phase of the study indicated significantly ($p < 0.05$) depressed levels of serum BDNF in all test groups when compared to those of the control group. Interestingly, Group 5 (codeine 30mg/kg treated) followed by Group 3 (tramadol 60mg/kg treated) had the lowest levels of the BDNF amongst the test groups, and the reduction in Group 5 was statistically significant ($p < 0.05$) when compared to the tramadol (60mg/kg) treated group.

Table 2. Changes in Hippocampus Levels of Brain-Derived Neurotrophic Factor (BDNF) following administration of some opioids in rats.

Groups	BDNF (ng/L) Levels		
	Acute phase (14 days)	Sub-chronic phase (28 days)	Chronic phase (56 days)
Group 1 control (distilled water)	114.00 ±3.20	101.66 ±2.75	81.93 ±2.29
Group 2 (tramadol 20mg/kg)	110.42 ±2.47	88.64 ±0.75 ^a	59.95 ±0.60 ^a
Group 3 (tramadol 60mg/kg)	98.29 ±3.89 ^a	85.64 ±2.06 ^a	51.05 ±3.34 ^{a,b}
Group 4 (codeine 10mg/kg)	96.82 ±3.17 ^a	81.52 ±1.21 ^a	67.21 ±0.57 ^a
Group 5 (codeine 30mg/kg)	92.90 ±1.80 ^a	76.09 ±2.41 ^{a, b}	61.47 ±3.46 ^{a, c}

Values are presented as Mean ± SEM, n= 5.

^a significant difference when compared to the mean value of group 1 (p<0.05)

^b Significant difference when compared to the mean value of group 2 (p<0.05)

^c Significant difference when compared to the mean value of group 3 (p<0.05)

^d Significant difference when compared to the mean value of group 4 (p<0.05)

The result in Table 2 above shows the changes in hippocampus levels of Brain Derived Neurotrophic Factor (BDNF) following administration of some opioids in rats. From the acute phase of the study, the BDNF levels in the hippocampus were found to be significantly (p<0.05) reduced in groups 3 (tramadol 60mg/kg), 4 (codeine 10mg/kg), and 5 (codeine 30mg/kg) when compared to those of the control group. The codeine treated groups had the lowest level of BDNF levels. At the sub-chronic phase of the study, all test groups had reductions in their BDNF levels when compared to those of the control group. The BDNF reductions in group 5 were also found to be significant (p<0.05) when compared to group 2 (tramadol 20mg/kg). From the outcome of the chronic phase of the study, all test groups indicated significant (p<0.05) reductions in their hippocampal BDNF level when compared to those of the control. Notably, the tramadol-treated groups (2 and 3) followed by those of codeine treatment (4 and 5) had the lowest levels of the marker.

4. Discussion

In response to the growing public health crisis of tramadol and codeine misuse globally, particularly in regions like Nigeria, this study aims to characterise the dose and time-dependent effects of these opioids on BDNF expression. The present study sought to evaluate the dose and time-dependent impact of these drugs on BDNF expression by quantifying alterations in serum and hippocampal BDNF levels in male Wistar rats subjected to acute, sub-chronic, and chronic administration.

Our data revealed consistent reductions in BDNF levels across all treatment groups compared to controls. The reductions were dose-dependent and time-dependent, with codeine

30mg/kg and tramadol 60mg/kg groups showing the lowest BDNF concentrations. Serum BDNF declined progressively across phases, while hippocampal BDNF suppression was most pronounced in chronic exposure, highlighting cumulative neurochemical disruption.

Opioids exert their primary effects through μ -opioid receptor activation, which inhibits adenylate cyclase activity and reduces intracellular cyclic AMP (cAMP) levels [6, 7]. As cAMP levels fall, there is a subsequent reduction in the activation of the cAMP-response element-binding protein (CREB), a transcription factor that is essential for the expression of the BDNF gene [23, 24]. The progressive decline in BDNF across the acute, sub-chronic, and chronic phases in this study suggests a persistent and cumulative suppression of the cAMP/CREB pathway. Also, chronic opioid exposure induces oxidative stress and mitochondrial dysfunction and hence an increase in reactive oxygen species (ROS) production, disrupts mitochondrial respiratory chain activity, and impairs neuronal survival and neurotrophic support [25, 26]. The BDNF supports mitochondrial integrity and antioxidant defences; hence, reduced BDNF further exacerbates oxidative damage, creating a cycle of neurotoxicity [27, 28]. The hippocampus, with its high metabolic demand and central role in learning and memory, is particularly vulnerable to oxidative injury and synaptic disruption. Mitochondria serve as the cell's primary energy generators, providing the ATP required for protein synthesis and synaptic remodelling. When opioids induce oxidative stress, they compromise mitochondrial membrane integrity and ATP production [25, 29]. This metabolic failure hinders the neuron's ability to maintain neurotrophic support, explaining why the chronic administration phase yielded the lowest BDNF concentrations in both the serum and the hippocampus.

Neuroinflammatory processes also contribute to opioid-induced BDNF suppression. Chronic opioid exposure

activates microglia and astrocytes, leading to the release of pro-inflammatory cytokines such as Tumour necrosis factor- α (TNF- α) and Interleukin-1-beta (IL-1 β), which downregulate BDNF synthesis and impair synaptic plasticity [30]. These inflammatory cascades promote synaptic pruning and neuronal apoptosis, further reducing hippocampal neurotrophic support.

Furthermore, gene regulatory modifications provide another possible mechanism by which opioids alter BDNF expression. Opioid exposure has been associated with histone deacetylation and deoxyribonucleic acid (DNA) methylation at BDNF promoter regions, leading to long-lasting repression of gene transcription [10]. Such changes may persist beyond drug exposure, contributing to enduring cognitive deficits. Furthermore, opioids disrupt hippocampal neurogenesis and synaptic remodelling. Physiologically, BDNF binds to Tropomyosin receptor kinase B (TrkB) receptors to facilitate neurogenesis and long-term potentiation (LTP), which is essential for dendritic spine formation and long-term potentiation (LTP), the cellular basis of learning and memory [5]. The severe reductions observed in hippocampal BDNF levels suggest a breakdown in these plastic processes. Because the hippocampus is particularly susceptible to oxidative damage and synaptic disruption, the lack of BDNF support likely leaves the brain vulnerable to accelerated neurodegeneration and cognitive decline. Reduced BDNF therefore impairs synaptic connectivity, consistent with the severe hippocampal suppression observed in chronic tramadol and codeine groups. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis may also play a role, as opioids elevate glucocorticoid levels, which suppress BDNF transcription and accelerate hippocampal atrophy [31].

Also, our data indicate that higher doses of opioids exacerbate the suppression of BDNF. This dose-dependency suggests that higher concentrations of codeine and tramadol lead to more intense receptor saturation and a more profound inhibition of neuroprotective signalling. The present findings of dose and time-dependent BDNF suppression align with established mechanisms of opioid neurotoxicity. Prior research shows that opioids like morphine inhibit the cAMP/CREB pathway, suppressing BDNF synthesis and maturation, which aligns with our observed progressive decline across exposure phases [23, 24]. Also, our observed dose-response effects also corroborate studies linking tramadol and codeine to oxidative stress and neurotrophic downregulation [25]. The critical role of exposure duration is underscored by studies showing neonatal morphine exposure alters hippocampal BDNF into adulthood [30] and also in patients on opioid maintenance therapy, associated with cognitive deficits [15] and neurodegeneration in other brain regions following chronic opioid exposure [26, 32]. Collectively, these convergent findings across models, doses, and timescales reinforce that BDNF suppression is a central, consistent feature of opioid-induced neurotoxicity, extending beyond abuse scenarios to prolonged therapeutic or moderate

misuse conditions. These significant reduction in BDNF observed here provides an experimental basis for the cognitive impairments and mood disorders often reported among chronic opioid users [10, 31].

5. Conclusion

The present study provides experimental evidence that tramadol and codeine significantly reduce BDNF levels in serum and hippocampal tissues of male Wistar rats. The reductions were dose-dependent and more pronounced with chronic exposure, suggesting that prolonged opioid use compromises neurotrophic support and synaptic plasticity. These findings underscore the significant risks associated with prolonged opioid therapy and highlight the need to develop neuroprotective strategies to safeguard brain health in individuals using or misusing opioids.

Abbreviations

BDNF	Brain-derived neurotrophic factor
TrkB	Tropomyosin receptor kinase B
NDLEA	National Drug Law Enforcement Agency
TNF α	Tumour necrosis factor- α
DNA	Deoxyribonucleic acid
LTP	Long-term potentiation
HPA	Hypothalamic pituitary adrenal
cAMP	Cyclic Adenosine Monophosphate
CREB	Cyclic AMP Response Element-Binding Protein
ATP	Adenosine Triphosphate

Conflicts of Interest

The authors declare no conflict of interest.

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