

Neurofilament Light Chain as a Biomarker of Oxidative Stress-related Neuronal Injury in Methamphetamine Addiction

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ABSTRACT

Background: Crystal methamphetamine addiction is associated with severe neurotoxicity and oxidative stress. **Objectives:** This study aimed to evaluate sera Neurofilament Light Chain as a label of neuronal injury and to estimate oxidative stress through total oxidant status and total antioxidant status in male and female crystal methamphetamine addicts before and after 21 days of treatment. **Methods:** Eighty individuals were included in the study, comprising 60 addicts and 60 healthy controls. Blood samples were collected from addicts before treatment and again after 21 days of treatment. Serum levels of Neurofilament Light Chain were measured by ELISA, while TOS and TAS were determined spectrophotometrically analyses. **Results:** The results illustrated significantly elevated Neurofilament Light Chain and TOS concentration ($p \leq 0.001$), and decreased TAS levels ($p \leq 0.001$) in addicts compared with healthy controls. After 21 days of treatment, Neurofilament Light Chain and TOS levels decreased significantly while TAS increased. Significant correlations were observed between oxidative stress parameters and neuronal injury, which may indicate a possible mechanistic link between neurotoxicity and oxidative imbalance in methamphetamine addiction. ROC curve analysis showed that Neurofilament Light Chain exhibited high diagnostic accuracy in distinguishing addicts from healthy subjects. **Conclusions:** These findings indicate that Neurofilament Light Chain and oxidative stress biomarkers may be useful indicators of methamphetamine-induced neurotoxicity and recovery following treatment.

ARTICLE HISTORY

Received March 13, 2026

Accepted April 25, 2026

KEYWORDS

Biomarker, Clinical Biochemistry, Methamphetamine, Neurofilament Light Chain, Oxidative Stress

1. Introduction

Addiction is one of the most widespread health and social problems in recent times in Iraqi society. Drug addiction is a compulsive dependence on a narcotic chemical that affects the brain, thereby impacting all cells and systems in the body, rendering the individual unable to stop using it despite being aware of its harmful consequences (Turan et al., 2023). Methamphetamine addiction is a major global health concern due to its profound neurotoxic effects on the central nervous system (Kulaksızoğlu et al., 2025; Omidvari et al., 2023). Chronic exposure to methamphetamine results in structural and functional alterations in the brain, including neuronal degeneration and cognitive impairment (Omidvari et al., 2023). One of the principal mechanisms underlying methamphetamine-induced neurotoxicity is oxidative stress (Jung & Damoiseaux, 2024; Turan et al., 2023). Methamphetamine stimulates excessive dopamine release and increases the production of reactive oxygen species, which can lead to lipid peroxidation, mitochondrial dysfunction, and neuronal damage.

In recent times, Neurofilament Light Chain has appeared as a sensitive biomarker for axonal injury (Bar-Or et al., 2025; Ebrahimi et al., 2025; Maceski et al., 2025; Rashidi et al., 2024; Sundar et al., 2022). Neurofilaments (NfL) are structural proteins located in neuronal cytoskeletons, and their release into circulation reflects neuronal damage (Ebrahimi et al., 2025; Jung & Damoiseaux, 2024). In parallel, oxidative stress biomarkers such as Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) provide an integrated assessment of the oxidant-antioxidant balance in biological systems

(Leyrer-Jackson et al., 2019; Shafi et al., 2025; Zhu et al., 2025). Despite growing evidence regarding methamphetamine-induced oxidative damage (Saloner et al., 2019), limited studies have investigated the relationship between neuroaxonal injury and oxidative stress markers in crystal methamphetamine (CM) addicts, particularly regarding treatment outcomes and gender differences (Hammood et al., 2025). Therefore, this study aimed to evaluate serum NFL, TOS, and TAS levels in male and female crystal methamphetamine addicts before and after 21 days of treatment.

2. Materials and Methods

2.1 Study Design

A total of blood samples was collected from a healthy control group (n=60) and a CM-addicted group (n=60). The healthy group consisted of 30 females and 30 males, aged 19 to 34 years. The CM-addicted group consisted of 30 females and 30 males, aged 17 to 36 years. Diagnostic procedures were conducted under the supervision of qualified physicians at Al-Ataa Hospital for Addiction Treatment and the Al-Qanal Center for Social Rehabilitation–Medical City Department. The sample collection and clinical assessments were conducted six months, from November 2024 to April 2025.

To check the effect of treatment, the same parameters were followed in both previously mentioned groups after 21 days of treatment. The treatment included psychotherapy, pharmacological interventions, and supportive counseling. Characteristics of the four groups are presented in Table 1.

Table 1: The Demographic Characteristics of the Four Groups (n=60 in Each Group)

Characteristics	Control Group		CM-Addicts Group		P-Value
	Female	Male	Female	Male	
Age (Year)	27.47 ± 7.47	26.13 ± 8.15	27.13 ± 8.00	27.01 ± 8.10	0.682 ^{ns}
Duration of Addiction (Years)	—	—	1.54±0.29	2.65±1.2	0.042*
BMI (Kg/M²)	23.6 ± 3.92	24.81 ± 3.19	23.39 ± 6.21	24.15 ± 6.62	0.491 ^{ns}
Family History					
Yes	0(0%)	0(0%)	4(6.6%)	6(10%)	< 0.001**
No	30(100%)	30(100%)	56(93%)	54(90%)	
Smoking					
Yes	8(26%)	13(43%)	15(50%)	20(66.6%)	0.163 ^{ns}
No	22(73%)	17(56%)	15(50%)	10(33.3%)	
History of Treatment					
Yes	0(0%)	0(0%)	30(100%)	30(100%)	0.971 ^{ns}
No	30(100%)	30(100%)	0(0%)	0(0%)	

Categorical variables are presented as numbers (percentages), and continuous variables are presented as mean ± SD. **

Refer to the p-value ≤ 0.0001, * Refer to the p-value ≤ 0.05, And ns was considered a non-significant difference.

2.2 Ethics Considerations

This study was conducted in full compliance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the relevant Institutional Review Board at the University of Baghdad College of Science (November 6, 2024, Ref.: CSEC/1124/0087) before the commencement of the study. All participants provided written informed consent after receiving detailed information about the study's purpose, procedures, and their rights, including assurances of confidentiality and privacy.

2.3 Blood Collection

A total of 5 mL venous blood samples were drawn from healthy participants (n=60) and the addicted participants before treatment (n=60) and after 21 days of behavioral and medical treatment. The collected blood was placed in a gel tube containing a clot activator (for serum collection).

2.4 Inclusion Criteria

The participants (≥18 years) who were current crystal methamphetamine users were included in the study. Participants who had a history of regular drug use for at least six months, as confirmed by self-report and positive toxicological screening, were included in the study. All subjects provided informed consent before sample collection.

2.5 Exclusion Criteria

The patients and the control individuals who had severe psychiatric disorders, chronic diseases affecting biological results, those who were using any medications, pregnant or breastfeeding women, and those who were unable to comply

with study protocols were also excluded.

2.6 Methods

1. Measurement of Neurofilament Light Chain

Serum NfL levels were determined using ELISA kits according to the manufacturer's instructions.

2. Measurement of Total Oxidant Status

Serum TOS was measured as described by Erel spectrophotometrically based on the oxidation of ferrous ions to ferric ions in the presence of oxidant species (Ertürk et al., 2024).

3. Measurement of Total Antioxidant Status

Serum TAS was measured using a colorimetric assay that evaluates the cumulative antioxidant capacity of the serum (Ertürk et al., 2024).

2.7 Statistical Analysis

All statistical analyses were performed using the SPSS program, version 27, under Windows (Statistical Package for the Social Sciences, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of differences between mean values was estimated by an independent T-test, one-way analysis of variance (ANOVA), paired samples test, Pearson correlation analysis, and ROC curve analysis; P-value ≤ 0.05 was considered significant, and P-value ≤ 0.001 was considered highly significant.

3. Results

Comparison between the studied groups (CM addicts and healthy controls) the results are observed in Table 2.

Table 2: Comparison of Serum NfL, TOS, and TAS in Sera of the Studied Groups (n=60 in Each Group)

Parameter	Control Group (mean $\pm$ SD)	Addicts Group (mean $\pm$ SD)	Post treated Addicts (mean $\pm$ SD)	P-Value
Neurofilament Light Chain pg/ml	9.95 $\pm$ 0.26	23.09 $\pm$ 0.98 ^a	19.15 $\pm$ 0.45 ^b	< 0.001 ^{a, b}
TOS (μ mol H ₂ O ₂ Eq/L)	7.62 $\pm$ 0.62	16.53 $\pm$ 0.74 ^a	10.91 $\pm$ 0.51 ^b	< 0.001 ^{a, b}
TAS (μ mol vit.C/L)	1430 $\pm$ 122	994 $\pm$ 30 ^a	1241 $\pm$ 48 ^b	< 0.001 ^{a, b}
OSI	0.62 $\pm$ 0.02	1.57 $\pm$ 0.03 ^a	1.02 $\pm$ 0.02 ^b	< 0.001 ^{a, b}

(a): indicated significant difference between the Addicts group and Control (Independent sample T-test), (b): indicated significant difference between Addicts before treatment and Addicts after treatment (Paired sample T-test).

From the above results (Table 2), it is clear that serum NfL levels showed a highly significant increase ($p \leq 0.001$) in CM addicts before treatment compared with healthy controls. Similarly, TOS and OSI levels showed a highly significant increase ($p \leq 0.001$) in addicts. In contrast, the levels of TAS showed a highly significant decrease ($p \leq 0.001$) in addicts compared with controls. The result agrees with the observations reported by Turan et al. (2023), who demonstrated elevated oxidative stress in Turkish individuals who used methamphetamine.

Following 21 days of treatment, the results showed a statistically significant decrease ($p \leq 0.001$) in the levels of NfL, TOS, and OSI compared with their corresponding values before treatment. On the other hand, despite this improvement, the values of NfL, TOS, and OSI remained highly significant increase ($p \leq 0.001$) than those observed in the control group. Conversely, TAS showed a highly significant increase ($p \leq 0.001$) after the treatment period compared with pre-treatment levels. However, TAS levels did not fully reach the levels detected in healthy controls.

To check if the sex has any influence on the measured parameters in the addict group. The results are presented in Table 3.

Table 3: Comparison of serum NfL, TOS, and TAS in sera of the addict groups according to sex differences (n=30 in each group).

Parameter	Females Addicts Group (mean $\pm$ SD)	Males Addicts Group (mean $\pm$ SD)	P-Value (Tow-tail)
Neurofilament Light Chain (Pg/ml)	25.15 $\pm$ 1.64	21.77 $\pm$ 1.14	< 0.001**
TOS (μ mol H ₂ O ₂ Eq/L)	18.30 $\pm$ 1.38	16.08 $\pm$ 1.25	< 0.001**
TAS (μ mol vit.C/L)	929 $\pm$ 17.08	994 $\pm$ 13.17	< 0.001**
OSI	1.97 $\pm$ 0.04	1.57 $\pm$ 0.01	< 0.001**

** Refer to the p-value ≤ 0.001 .

The results presented in (Table 3), NfL, TOS, and OSI showed a highly significant increase ($p \leq 0.001$) in females compared to males in the CM addicts' group, while TAS levels showed a highly significant decrease ($p \leq 0.001$) in the CM

female addicts compared to the CM male addicts. These results agreed with the results of Kulaksızoğlu *et al.*, in Turkey, who reported the oxidative stress in male individuals using paper impregnated with synthetic cannabinoids, compared with that in the Turkish healthy control (Kulaksızoğlu *et al.*, 2025).

To explore the relationship between oxidative stress and neuronal injury, Pearson's correlation detection was performed among the CM addicted individuals. The relationships between NfL and oxidative stress parameters, including TOS, TAS, and OSI. The results are presented in (Table 4).

Table 4: Comparison of serum NfL, TOS, and TAS in Sera of the CM addiction group (n=60).

Parameter	r	P-Value
NfL vs TOS	0.766**	< 0.001**
NfL vs TAS	-0.575**	< 0.001**
NfL vs OSI	0.557**	< 0.001**

** Refer to the p-value ≤ 0.0001

The Pearson correlation analysis showed highly significant associations between the studied parameters. A strong positive correlation was detected between NfL and TOS. In contrast, a moderated negative correlation was identified between NfL and TAS. Moreover, a positive correlation was observed between NfL and OSI.

To evaluate the diagnostic performance of NfL in recognizing CM addicts from healthy individuals, the receiver operating characteristic ROC curve analysis was performed. The ROC curve is illustrated in (Figure 1).

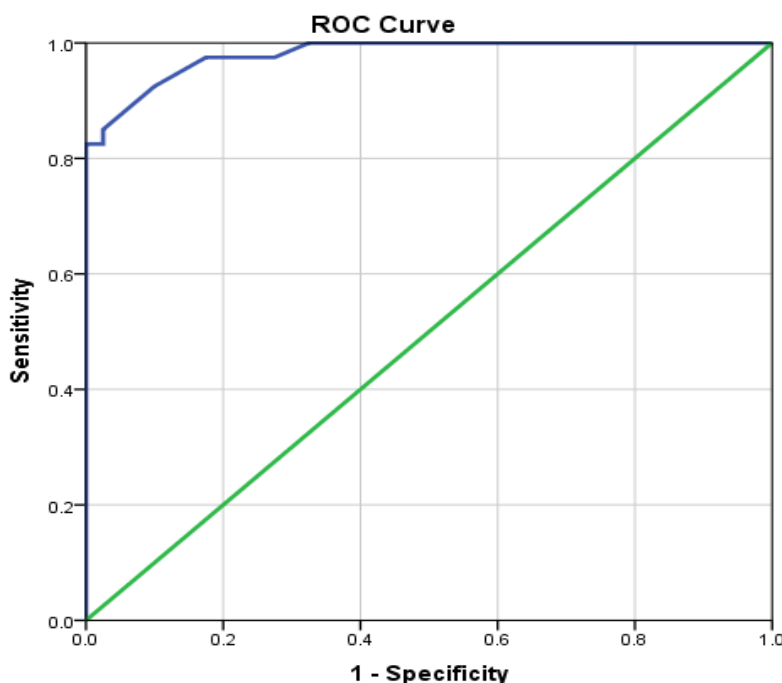


Figure 1: ROC Curve of NfL Level in CM Addicts and Control Groups

As shown in (Figure1), serum NfL concentration demonstrated a great diagnostic performance in identifying crystal methamphetamine addicts from healthy controls. The results observed from the above Figure are presented in (Table5).

Table 5: Measurements of AUC, P-value, Lower Bound, Upper Bound, Cut-Off Value, Sensitivity, and Specificity for NfL level in the CM addict group and the control group (n=60 to each group)

Parameter	AUC	SE	P-value	Asymptotic 95% CI		Cut-Off Value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Neurofilament Light Chain level	0.980	0.011	<0.001	0.958	1.000	22.00	93.3%	86.7%

The results (Table 5) showed that NfL a high area under the curve AUC. In addition, the optimal cut-off value demonstrated an acceptable balance between sensitivity and specificity.

To further investigate the relationship between oxidative stress and neuronal damage, linear regression analysis was

carried out to evaluate the role of oxidative stress on NfL concentrations among CM addicts. The results are presented in (Figure 2, Table 6).

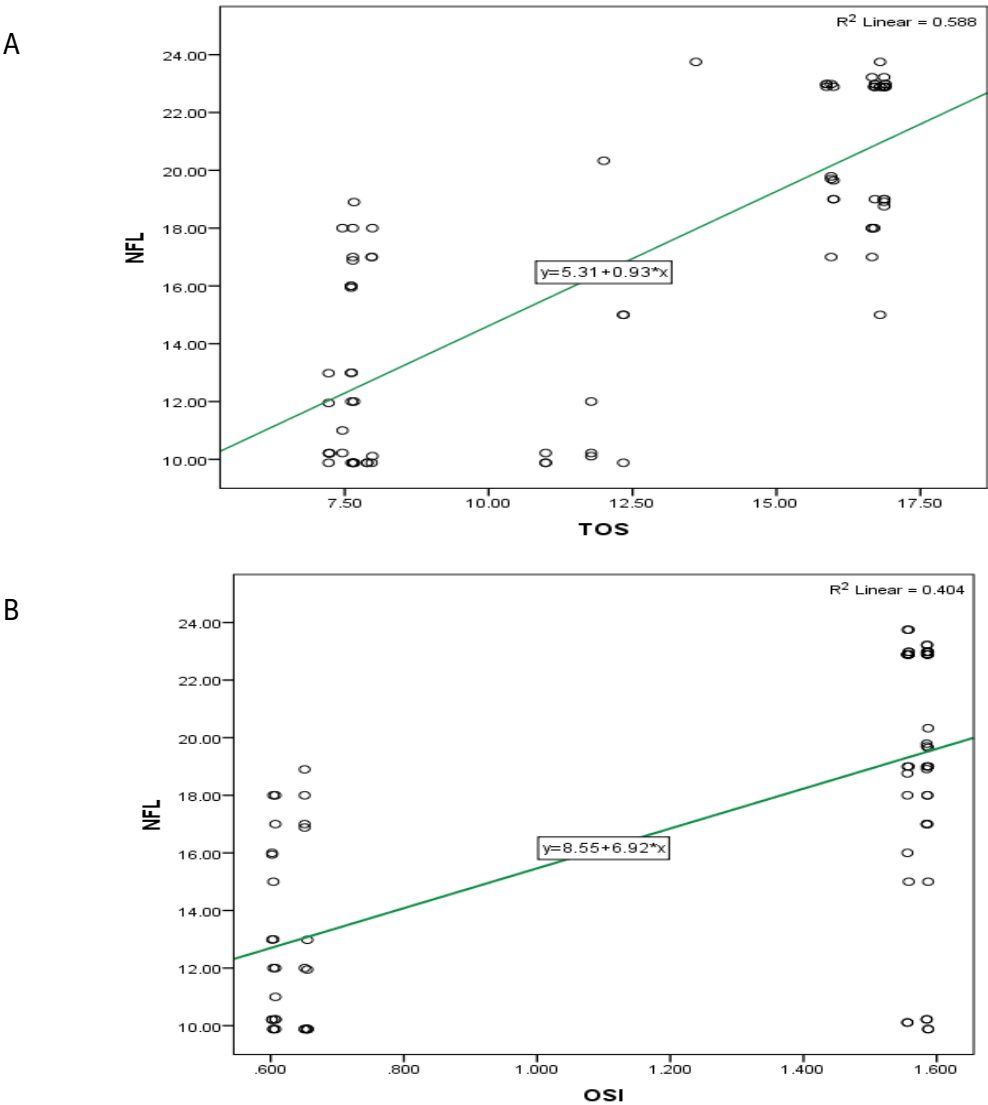


Figure 2: Linear Regression Plots Show the Relationship between Oxidative Stress and NfL Level in the CM Addict group. A: The Relationship between TOS and NfL level B: The relationship between OSI and NfL level

As shown from (Figure 2), the scatter plots illustrate a clear positive linear relationship between NfL level and both TOS and OSI.

Table 6: Multiple Linear Regression Analysis for TOS and OSI with Constant NfL Level in the CM Addict Group (n=60)

Parameters	Beta β	Standard Error	P-Value
TOS	0.687	0.140	<0.001
OSI	0.390	0.253	≤ 0.05
NfL	-	-	<0.001

The obtained results (Table 6) showed multiple liner regression analysis was performed the TOS a positive significant predicted of NfL at ($\beta = 0.687$, $p = 0.000$). Similarly, the OSI was significantly associated with increase NfL concentrations at ($\beta = 0.390$, $p = 0.047$). The model explained a considerable proportion of the variance in NfL ($R^2 = 0.592$).

4. Discussion

The investigation in the present study explored alterations in neuronal integrity and oxidative balance among individuals diagnosed with autism spectrum disorder. The results showed noticeable changes in circulating levels of NfL,

together with oxidative stress index changes, including TOS and TAS. These observations may indicate that disturbances in neuronal structural stability could be accompanied by an imbalance in redox homeostasis.

Elevated NfL concentration is generally interpreted as an indication of axonal injury or disorder of neuron structure (Bar-Or et al., 2025; Jung & Damoiseaux, 2024). In conditions of neurological and neurodevelopmental disorders, refined alterations in neuronal cytoskeletal organization may lead to the release of intracellular structural proteins into the bloodstream (Maceski et al., 2025). Therefore, the detection of an increase in concentration of NfL might provide indirect evidence of ongoing stress of the neuronal or reconstruction processes (Maceski et al., 2025). Similar observations were reported by Jung and Damoiseaux (2024), who demonstrated that increased NfL levels, which are associated with neuronal damage and neurodegenerative processes in patients with Alzheimer's.

At the same time, the oxidative stress observed in the current study suggests the increased oxidant generation combined with decreased antioxidant defense mechanisms among addicts. These results indicated that chronic exposure to methamphetamine may lead to excessive production of reactive oxygen species and impairment of antioxidant defense mechanisms (Han et al., 2025). Previous neurobiological investigations have shown that CM induces oxidative stress through dopamine auto-oxidation, mitochondrial dysfunction, and activation of neuroinflammatory pathways (Ramli et al., 2025). As Sundar et al. (2022) highlight, stimulant abuse can disrupt brain redox balance and contribute to neuronal injury, while Rashidi et al. (2024) demonstrated that dopaminergic toxicity associated with methamphetamine promotes oxidative damage within neuronal cells.

The improvement observed after 21 days of treatment provides additional insight into the dynamic relationship between oxidative stress and neuronal injury. Following treatment, these parameters NfL, TOS, TAS, and OSI, did not fully return to the levels observed in healthy individuals, suggesting that the recovery of integrated and oxidative balance requires a longer period of abstinence (Zhu et al., 2025).

Another important observation was the difference between the sexes of participants. Females, addicts demonstrated higher levels of OSI as well as increased concentration of neurofilament light chain compared with that of males. These observations may indicate that the biological sex could influence exposure to neuronal stress oxidation-reduction imbalance (Beauty et al., 2025; Hammood et al., 2025; Mirza et al., 2025). Hormonal aspects, metabolic variation, and differences in immune response have all been proposed as contributors to sex related variability in conditions of the neuron (Abreu Minero et al., 2022). The elevated oxidative stress index observed in the females further supports the presence of a higher imbalance between oxidant production and antioxidant capacity (Hammood et al., 2025). This observation might suggest that the females experience a relatively stronger oxidative load, which could contribute to enhanced neuronal stress and structural alterations.

The correlation analysis (Table 4) further supported the potential link between oxidative stress and neuronal injury. A strong positive correlation between NfL and OSI suggests that the increased oxidant production contributes to neuronal structural injury. In contrast, the negative correlation observed between NfL and TAS indicates that reduced antioxidant defense exacerbates neuronal vulnerability to oxidative damage. These findings support the hypothesis that oxidative imbalance plays a critical role in the neurotoxic effects associated with methamphetamine exposure (Omidvari et al., 2023).

The receiver operating characteristic analysis provided evidence regarding NfL as a biomarker. The ROC curve demonstrated that NfL possesses a good ability to discriminate between CM addicts and healthy controls. This diagnostic performance suggests that NfL may serve as a useful biomarker for the detection of neuronal injury associated with stimulant addiction. The diagnostic potential of NfL has also been reported in various neurological disorders, where elevated circulating levels were shown to reflect axonal degeneration and neuroaxonal damage (van Zeggeren et al., 2022; Zanella et al., 2022).

From the obtained result (Figure 2 and Table 6), the significant predictive effect of oxidative stress on NfL suggests that redox imbalance may directly affect cytoskeletal disruption and axonal degeneration. This is consistent with the mechanism proposed by Tarnacka, highlighting the oxidative stress-mediated neurotoxicity as a central pathway in stimulant-induced brain damage (Tarnacka et al., 2021).

Generally, the findings of the present study highlight that CM addiction is associated with pronounced oxidative stress and neuronal injury. The combined assessment of oxidative stress markers together with NfL may therefore provide valuable insights into the biological mechanisms underlying stimulant-induced neurotoxicity. Furthermore, monitoring these biomarkers may help in evaluating disease severity and tracking recovery during addiction treatment.

5. Conclusion

The study showed that the crystal methamphetamine addiction is associated with important neuronal damage and oxidative stress imbalance. Elevated serum NfL, TOS, and OSI levels, accompanied by decreased TAS levels, reflect neurotoxicity and impaired antioxidant defense mechanisms.

After 21 days of treatment, partial recovery was observed in NfL, TOS, TAS, and OSI levels. Therefore, these parameters did not return to such levels observed in healthy controls, suggesting that neuronal recovery and restoration of oxidative balance may require a long term of abstinence

6. Recommendation

Future studies involving a larger population and longer follow-up periods are recommended to further clarify the relationship between oxidative stress and neuronal damage in substance disorders.

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