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Original Article

DOSE-DEPENDENT EFFECTS OF METHAMPHETAMINE ON NEUROCHEMICAL AND NEUROIMMUNE MARKERS IN THE BRAIN, SPLEEN, AND KIDNEY

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ABSTRACT

Objective: This study evaluated the effects of low, moderate, and high methamphetamine (METH) doses on noradrenaline (NA), dopamine (DA), vesicular monoamine transporter type 2 (VMAT-2), and toll-like receptor 4 (TLR-4) in central and peripheral nervous systems(PNS) of lymphoid and non-lymphoid organs.

Methods: A total of 120 Balb/c male mice (8–9 w) were divided into four groups (n=30). Three groups received intraperitoneal METH injections (2 mg/g, 5 mg/g, 10 mg/g), while the control received saline. Subgroups (n=10) were sacrificed at 1 w, 2 w, or after a 7 days withdrawal. Brain central nervous system (CNS), spleen (lymphoid), and kidney (non-lymphoid) tissues were homogenized, and supernatants analyzed for DA, NA, VMAT-2, and TLR-4 using ELISA kits.

Results: NA, DA, VMAT-2, and TLR-4 levels significantly ($p<0.05$) increased in all METH-treated groups compared with control, in a dose-and time-dependent manner. After 7 d withdrawal, Group 1 (2 mg/g) values normalized, whereas Group 2 (5 mg/g) remained elevated versus control. All Group 3 (10 mg/g) mice died during withdrawal, because a high dose is synergistic central and peripheral toxicity.

Conclusion: Low-dose METH-induced alterations may resolve upon cessation, suggesting reversible effects. Moderate doses cause persistent damage requiring intervention, while high doses lead to severe withdrawal and mortality. Importantly, METH effects extend beyond the CNS to peripheral systems, impacting both lymphoid and non-lymphoid organs.

Keywords: METH, NA, DA, VMAT-2 and TLR-4

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INTRODUCTION

Methamphetamine's (METH) rapid spread across the blood-brain barrier (BBB) is made possible by its small size and high lipophilicity. Organic cation transporters that are expressed on BBB endothelial cells and use METH as a substrate [1], further enhance transport of METH over the BBB. Numerous neurological conditions have been linked to METH, a highly abused psychoactive stimulant. It has neurotoxic and degenerative effects due to a variety of modes of action, such as excessive release of neurotransmitters (NEUs), blockage of NEU uptake transporters, degradation of NEU receptors, oxidative stress, etc [2].

METH has a strong effect on the Dopamine DA system, which is one of the remarkable changes it causes in the neurological system. These changes are accompanied by notable alterations to cognition and behavior [3]. Normally, by removing DA from the synaptic cleft, dopamine transporter DAT terminates the neurotransmitter's actions. However, when METH binds to DAT, it interferes with extracellular DA reuptake and causes DA to be transported out of the cell in reverse, which raises the concentration of DA in the synaptic cleft [4, 5]. The stimulant and inhibitory effects of the medicines determine all neurotransmitter levels in the addiction. Neurotransmitters and drugs primarily target receptors to demonstrate their effects [6].

Methamphetamine's neurotoxic effects cause changes in DA levels, with higher levels being associated with a subsequent decrease in DA neurotransmission. On the other hand, methamphetamine-induced neurotoxicity decreases by DA receptor antagonists or synthesis inhibitors. Repeated METH injection lowers striatal concentrations of DA and DA metabolites in many brain regions [7]. In addition to causing general DA depletion, a single dose of MA also causes the production of the reactive nitrogen species peroxynitrite, a free radical created when superoxide and nitric oxide interact. Moreover, it has been shown that superoxide plays a role in MA's DA depleting effects since overexpressing a copper-zinc superoxide dismutase protected against both peroxynitrite production and MA-induced DA depletion [8]. Methamphetamine increases extracellular dopamine and norepinephrine by transporter reversal, which modifies catecholamine dynamics. Renal tissue damage is exacerbated by the consequent increase in cytosolic monoamines, which encourages oxidative stress and cellular malfunction [9].

According to several neurotoxic models, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, the noradrenaline (NA) system is generally neuroprotective and affects the vulnerability of nigrostriatal dopaminergic neurons [10]. The plasma membrane NA transporter (NET), which is expressed by NA neurons and has the ability to absorb METH, has been proposed as a "buffer" that can sequester toxins like METH [11]. Furthermore, alterations in the release of possible neuroprotective noradrenergic co-transmitters, including neuropeptide Y and galanin are probably brought on by damage to NA neurons. Therefore, it is still unclear if endogenous NA plays a special role [12].

As one of the primary NEUs that generate reward, NA nuclei contribute to the neurobiology of reward produced by MA. NA also strongly modifies METH-induced behaviors and reward route, which form the basis for behavior leading to MA addiction. Because genetic polymorphisms affect the limiting enzyme for NA synthesis, DA beta-hydroxylase, which is implicated in substance abuse disorders, NA neurons are also implicated in genetic susceptibility to MA-induced behavioral consequences [13], additionally, selective genetic or pharmacological blockage of NA synthesis was found to mimic the effects of METH-induced gliosis, DA terminal damage, extracellular ROS production, striatal DA release, and the creation of autophagic vacuoles and multilamellar whorls in intrinsic striatal neurons. These findings show that METH's toxicity to the DA system is increased when endogenous NA is absent [14].

Vesicular monoamine transporter 2 (VMAT 2) is an essential membrane protein that transports dopamine (DA) from the cytosol into synaptic vesicles, and it plays a crucial role in METH-induced

DA release [15]. After neurotransmitter production and/or clearance from the extracellular environment, VMAT2 translocate monoamines from the cytosol across the vesicle membrane into the vesicle lumen within the presynaptic terminals of monoaminergic neurons [16]. ATPase, a proton pump, is strongly linked to this transport process. DA leaks from the vesicle into the cytosol when METH, a "weak base," upsets the proton gradient on the two sides of the vesicle, which is necessary for DA sequestration [4]. Because METH's structure is similar to that of dopamine, it can compete with VMAT2, disrupt the pH gradient required for dopamine transport into synaptic vesicles [17], and stimulate the reserpine binding site on VMAT2 to stop additional dopamine vesicular packaging [18]. Additionally, VMAT 2's density and functioning on the cellular membrane may be reduced by METH [1]. When VMAT2 is disrupted or dysregulated (for example, by medicines, toxins, or genetic variations), vesicular monoamine storage is compromised, cytosolic monoamine concentrations rise, and oxidative stress and cellular dysfunction may result [19]. Methamphetamine causes immunosuppression and decreases splenic lymphocyte function, which may be a factor in the increased risk of infections among methamphetamine abusers [20]. Methamphetamine enhances extracellular levels of monoamines, especially dopamine and norepinephrine, by interfering with VMAT-2-mediated vesicular storage and reversing the function of their plasma membrane transporters. These processes can also take place in peripheral tissues that express dopaminergic system components, therefore they are not limited to the central nervous system [21].

The interplay between the immunological and neurological systems is well-known [22]. The transmemberane receptor known as Toll-like receptor-4 (TLR4) is essential for the innate immune response [23]. Its downstream signaling pathways are also important for the development and progression of neurodegenerative disease because they cause the microglial cells to remain activated, which raises pro-inflammatory mediators and reactive oxygen species, which in turn cause cellular macromolecular damage and apoptosis [24]. Nevertheless, it is unclear if TLR4 contributes to METH-induced microglial toxicity, indicating that METH reduces TLR4 expression and distribution on the surface of cells that resemble microglia. In addition, METH alters the morphology of cells, disrupts TLR4 signaling pathways, and lowers the production of pro-inflammatory mediators by microglia [25]. Following endotoxin incubation, METH also reduces the distribution and expression of TLR4 receptors on the surface of cells that resemble microglia [25].

This study aims to assess the effects of low dose (2 mg/g), moderate dose (5 mg/g), and high dose (10 mg/g) of METH on the levels of NA, DA, VMAT-2 and TLR-4 in the brain (as central nervous system), spleen (as lymphoid organ) and kidney (as Non-lymphoid organ)

MATERIALS AND METHODS

Chemicals and reagents

Phosphate buffer saline (PBS), Protease inhibitor, Major chemical like (DA) Cat. No: QS0190Mo; Cat. No(NA):RE10132; (VMAT-2) Cat. No: RE3340M; (TLR4) Cat. No: RE2838M, was procured from Reedbiotech/china.

Animals

One hundred and twenty Balb/c male mice aged 8 to 9 w, obtained from the Iraqi Center for Cancer Research and Medical Genetics/Baghdad/Iraq, were used in this study. Every mice (five per cage) was housed in a controlled setting with a 12 h light-dark cycle and unlimited access to food as well as water. The institutional ethical committee of the College of Medicine at Ibn Sina University of Medical and Pharmaceutical Sciences (ISU.14.2.26), where the study was carried out, reviewed and approved all animal-related experimental procedures. These procedures were carried out in compliance with globally recognized standards for the care and use of laboratory animals.

Before the experiment began, the experimental animals were housed in standard settings (temperature 22±2 ºC, 12 h light/12 h dark cycle), with unrestricted access to a standard pellet meal and unlimited water. They were also acclimated to laboratory conditions for at least 7 d.

Methamphetamine

METH provided by from the Ministry of Interior/Criminal Laboratories Department under official authorization permit (no.7010, date: 15/10/2023). Methamphetamine was administered as the hydrochloride salt, The powdered substance was dissolved in sterile 0.9% saline immediately before usage.

Animals groups and methamphetamine exposure

The animal were divided into four groups (n=30 mice). Three groups treated with 100µl METH by intraperitoneal injection. The METH were set at low dose 2 mg/g, moderated dose 5 mg/g and high dose 10 mg/g according to previous studies [26, 27]. The first group was treated with low concentration of METH, 2 mg/g daily. The second group was treated with moderate concentration of METH; 5 mg/g daily. The third group was treated with high concentration of METH, 10 mg/g daily. The last group treated with normal saline and served as control group.

The mice were sacrificed by cervical dislocation at different time-points, 10 mice from each group after 7 d of drug use, 10 mice from each group after 14 d of drug use and then the remain mice left without treated for 7 d before sacrificed them. However, all the mice treated with high concentration of METH die after leaving them 7 d without drug. The right part of spleen (lymphoid organs) and brain (central nerve system) and the right kidney (Non-lymphoid organs) were put in tubes had phosphate buffer saline (50 mmol)PBS; final composition 50 mmol phosphate, pH 7.4, 137 mmol NaCl, 2.7 mmol KCl, 1 mmol EDTA, and 0.1% Triton X-100and immediately frozen at-20 °C until used. The right part of spleen, brain, and the right kidney were put in tubes had phosphate buffer saline (50 mmol)PBS; final composition 50 mmol phosphate, pH 7.4, 137 mmol NaCl, 2.7 mmol KCl, 1 mmol EDTA, and 0.1% Triton X-100 and immediately frozen at-20C until used.

The frozen tubes were left in room temperature to melt and the tissue was placed on filter paper to absorbed excess water. The part of organs were weighted using an analytical balance (ShimadzuAY220, Shimadzu Corporation, Japan) and transferred to 1,5 ml Eppendorf cups. Protease inhibitor (100x PIC; Solarbio life science, Beijing; china; Cat. No. P6730;LotNo.2312005;MFD: 27/12/2023) was diluted in buffer saline 1% and add to sample at a relation 100 mg tissue per 1 Ml for parameters determination. The tissues were disrupted using an ultrasonic cell disruptor (Sonics vibra-cell VCX130, Sonics and Materials Inc. USA) for 5-10 sec set at maximal power. Sonicated samples were centrifuged (Eppendorf 5804, Eppendorf, Germany) at 20,000 × g at 4 °C for 10 min. Supernatants were removed, a liquoted, and stored at-80Cº until used.

The samples were used to estimate the level of Dopamine (DA), Noradrenaline (NA), The Vesicular Monoamine Transporter type 2 (VMAT-2) and Toll-Like Receptor 4 (TLR-4) by Sandwich enzyme-linked immunosorbent assay (ELISA) using the kits commercially available from Reed biotech/china An ELISA microplate reader (BioTek, USA) was used to measure absorbance. All sample absorbance levels fell within the linear dynamic range of the standard curves, and all ELISA experiments were carried out precisely in accordance with the manufacturer's instructions.

Statistical analysis

Results were expressed as mean±SE. The data was examined for many comparisons after one-way analysis of variance (ANOVA), using Fisher's test. A regression analysis based on analysis of combined variance was done (ANCOVA). Stat View 5.0 was used to conduct all the experiments data analysis. The differences were considered significant when $p<0.05$.

RESULTS

The levels of Dopamine (DA), Noradrenaline (NA), Toll-Like Receptor 4 (TLR-4) and Vesicular Monoamine Transporter 2 (VMAT-2) were determined in the brain, spleen and kidney tissues of Mice groups treated with METH and control group sacrificed in different time points, after one week and two weeks of drug use and one week after drug use stopping.

The table 1: showed in brain, NA and DA levels significantly increased in all groups after one week of METH use compared to control. However, the differences of these levels were significant (p≤0.05), the highest was in the group 3, treated with the highest METH concentration, and the lowest was in the group 1, treated with the lowest METH concentration. Moreover, there was no significantly differences in DA levels at end of treatment and 1 in all groups while there were significantly differences between its levels in the groups treated with METH compared to the control, After withdrawal drug use stopping, the levels of NA and DA in the group 1 returned to normal as same as in the control, but their levels still significantly higher than the control in the group 2. Within this week all the mice in the group 3 died.

The level of TLR-4 and VMAT-2 significantly increased in the group 3 compared to other drug use groups and the control while their levels in the group 1 and 2 had no significantly differences compared to the control, in all time-points. While there was no difference in the level of TLR-4 and VMAT-2 in the group 1, 2 and the control between time-points, VMAT-2 level in the group 3 significantly increased in 7 d compared to one

In the table, 2 showed comparable results were obtained in the spleen significantly (p≤0.05) increased in all groups during and end of treatment of METH using compared to the control. However, the differences between these levels were significant, the highest was in the group 3 and the lowest was in the group 1. After withdrawal, drug use stopping, the levels of NA and DA in the group 1 returned to normal as same as its levels in the control, but their levels in the group 2 still significantly higher than control. Within this week all the mice in the group 3 died. NA levels after during treatment of drug stopping was significantly higher in the group 2 and lower in the group 1 while DA levels was significantly lower in both groups (1and 2) compared to end of treatment of drug using.

There was no significantly difference in the level of TLR-4 and VMAT-2 between the group 1 and control in all time-points, their levels were significantly higher in the group 2 and 3, which were the highest, compared to the control. Between the time-points, both the level of TLR-4 and VMAT-2 were significantly higher after end of treatment of drug use compared to during treatment in the group 3 while TLR-4 level was significantly higher during treatment after drug stopping compared to during and end of treatment use in the group 2. In table 3, NA levels significantly (p≤0.05) increased in all groups during of treatment use compared to the control. These differences were significant, the highest was in the group 3 and the lowest was in the group 1. While there were no significantly differences between NA levels at end of treatment using than during of treatment in the group 2 which still significantly higher than the control, its levels significantly reduced in the group 1 which there was no significant difference than the control, and in the group 3 which still significantly higher than the control. After withdrawal, during of treatment, the levels of NA in the group 1 returned to normal as same as its levels in the control, but their levels in the group 2 still significantly higher than the control. Within this week all the mice in the group 3 died.

In the table 6 showed essentially the same differences were observed in the kidney. While there was no significantly difference in the level of TLR-4 and VMAT-2 between the group 1 and control in all time-points, their levels were significantly higher after end of treatment use in the group 2 and 3, which were the highest, and one week after drug stopping compared to the control. Between the time-points, both levels of TLR-4 and VMAT-2 were significantly higher after two weeks of drug use compared to during of treatment in the group 2 and 3 as well as end of treatment after drug stopping in the group 2 compared to during of treatment after drug use. However, there was no significantly difference in these parameters of the group 1 between all time-points.

The correlations results

While in the table 3, A significant positive connection was found between TLR-4 and VMAT-2 (P=0.02, R=0.22). Additionally, TLR-4 demonstrated a moderately significant connection with DA (P <0.0001, R=0.52). Furthermore, TLR-4 and NA showed a small but significant connection (P=0.007, R=0.3). No significant correlations were found between NA (pg/ml) and the measured parameters (P=0.17, R=0.14) and (P=0.54, R=0.06), indicating no significant relationship. With a significant moderate-to-strong positive correlation with one of the variables (P <0.0001, R=0.6), VMAT-2 (pg/ml) showed the strongest correlation in the dataset, suggesting a physiologically relevant association.

Table 1: The levels of dopamine (DA), Noradrenaline (NA), Toll-like receptor 4 (TLR-4) and Vesicular monoamine transporter 2 (VMAT-2) in Brain tissue of all groups at during and end treatment of METH use and after withdrawal

G	DA DT	DA ET	DA AW	NA DT	NA ET	NA AW	VMAT-2 DT	(VMAT-2 ET	VMAT-2 AW	TLR-4 DT	TLR-4 ET	TLR-4 AW
G1	19.1±1.4c	20.5±0.9b	18.2±1.5b	765±89c*	399±50c#	268±43b#	364±55b	358±29b	319±51	0.224±0.03bc	0.228±0.02bc	0.190±0.04b
G2	25.6±1.3b	23.7±0.7b	26.7±0.5a	1024±121b	728±91b	626±103a	518±7.9b	353±91b	363±41	0.408±0.04b	0.505±0.06b	0.413±0.03a
G3	31.7±0.7a	28.7±1.8a	---	1320±76a	1436±107a	---	3298±206a*	1210±233a#	---	1.297±0.28a	1.467±0.36a	---
C	14.8±1.7d	16.1±1.5c	18.6±1.7b	205±15d	297±57c	217±27b	320±33b	314±20b	316±31	0.167±0.05c	0.142±0.03c	0.157±0.04b

During treatment the(DT): the first METH use week, End of treatment(ET): the second METH use week, After withdrawal(AW) one week after METH use stopping, Group(,G),Control(C). Value are expressed as mean±SD; n = 7–8 animals per group (total n = 30). Different small letters indicate significant differences among groups (p<0.05), and different symbols (*, #, $) indicate significant differences between time-points.

Table 2: The levels of dopamine (DA), Noradrenaline (NA), Toll-like receptor 4 (TLR-4) and vesicular monoamine transporter 2 (VMAT-2) in spleen tissue of all groups at during and end treatment of METH use and after withdrawal

G	DA DT	DA ET	DA AW	NA DT	NA ET	NA AW	VMAT-2 DT	VMAT-2 ET	VMAT-2 AW	TLR-4 DT	TLR-4 ET	TLR-4 AW
G1	16.0±1.2b*	14.6±3.0c*	9.1±1.3b#	770±88b*	495±65c#	316±27b#	115±9.2b	77±13c	55±2.2b	0.838±0.12b	0.805±0.15c	0.969±0.29b
G2	20.0±0.4ab*	22.6±3.0b*	15.4±2.1a#	914±64b*	1181±105b#	1689±205a$	201±29b	162±44b	139±14a	0.890±0.09b*#	1.349±0.27b*#	1.458±0.25a#
G3	27.5±1.6a	24.2±1.5a	---	1281±110a	1484±295a	---	449±55a*	782±107a#	---	1.122±0.07a*	2.179±0.19a#	---
C	8.8±2.0c	9.1±1.3d	8.9±0.7b	224±36c	250±50d	289±76b	55±9.8c	50±6.5c	52±2.5b	0.652±0.09c	0.525±0.13d	0.782±0.24b

During treatment the (DT): the first METH use week, End of treatment(ET): the second METH use week, After withdrawal(AW) one week after METH use stopping, Group(,G), Control(C), value are expressed as mean±SD; n = 7–8 animals per group (total n = 30). Different small letters indicate significant differences among groups (p<0.05), and different symbols (*, #, $) indicate significant differences between time-points.

Table 3: The levels of dopamine (DA), Noradrenaline (NA), Toll-like receptor 4 (TLR-4) and vesicular monoamine transporter 2 (VMAT-2) in kidney tissue of all groups at during and end treatment of METH use and after withdrawal

G	DA DT	DA ET	DA AW	NA DT	NA ET	NA AW	VMAT-2 DT	VMAT-2 ET	VMAT-2 AW	TLR-4 DT	TLR-4 ET	TLR-4 AW
G 1	16.1±2.7b	16.7±1.3c	13.1±1.3b	462±102b*	88±19c#	63±16b#	410±150*	1093±219c#	882±187b#	0.816±0.31	0.930±0.53c	1.033±0.40b
G 2	19.0±1.2a	18.9±0.8bc	17.0±1.5a	572±92b	335±98b	558±96a	591±136*#	2713±237b#	2771±599a#	1.100±0.33*	3.744±0.19b#	4.327±0.38a#
G 3	21.2±3.3a	23.9±1.5a	---	1106±175a*	724±39a#	---	634±0.09*	3864±126a#	---	1.174±0.36*	4.410±0.29a#	---
C	10.0±0.5b	10.4±0.2c	11.2±0.5b	54±9.5c	56±5.2c	64±8.5b	331±35	509±100c	610±97b	0.554±0.09	0.610±0.10c	0.680±0.17b

During treatment the (DT): the first METH use week, End of treatment(ET): the second METH use week, After withdrawal(AW) one week after METH use stopping, Group (,G),Control(C). Value are expressed as mean±SD; n = 7–8 animals per group (total n = 30). Different small letters indicate significant differences among groups ($p<0.05$), and different symbols (*, #, $) indicate significant differences between time-points.

Table 4: The correlation of noradrenaline (NA) and dopamine (DA) levels with the levels of toll-like receptor 4 (TLR-4) and vesicular monoamine transporter 2 (VMAT-2)

	TLR-4 (ng/ml)	VMAT-2 (pg/ml)	NA (pg/ml)
DA (pg/ml)	P=0.02, R=0.22	P=0.007, R=0.3	P<0.0001, R=0.52
NA (pg/ml)	P=0.17, R=0.14	P=0.54, R=0.06	----------
VMAT-2 (pg/ml)	P<0.0001, R=0.6	----------	----------

DISCUSSION

Methamphetamine is a potent central nervous system (CNS) stimulant that works pharmacologically and behaviorally by altering the brain's dopaminergic reward circuitry, which is widely thought to be the cause of the rewarding effects of drugs of abuse [28]. METH has serious neurotoxic effects and is a highly addictive psychostimulant substance (4). According to Jayanthi *et al.,* (2021), long-term METH dependency damages neurons and lowers cognitive function, memory, and attention. METH is a lipophilic medication that readily penetrates the blood-brain barrier (BBB) [7]. METH also changes the expression of adhesion and tight junction molecules and compromises the integrity of the BBB [3].

The first major findings of our research were the DA and NA levels were higher in the METH-exposed brain than in the control. This result is consistent with several other research that indicate methamphetamine's effect on monoamine neurotransmitters. The main way that methamphetamine (METH) acts in the brain is by encouraging the release of extracellular monoamine neurotransmitters from nerve terminals, such as dopamine (DA) and norepinephrine (NA) [8, 13]. A human Dopamine and other catecholamines are found in significant quantities in Tregs, which also constitutively express TH. Tregs are able to store catecholamines in vesicular storage because they also express VMAT-1 and VMAT-2 [29]. The release of extracellular monoamine neurotransmitters, such as dopamine (DA) and norepinephrine (NA), interacts with vesicular monoamine transporter-2 (VMAT-2) and toll-like receptor 4 (TLR4) [7]. The main role of VMATs is to confine neurotransmitters in vesicles, which is an essential step in controlling neurotransmitter release [16]. This evidence could explain the elevated levels of VMAT-2 and TLR-4 found in this work. On the other hand, VMAT2 was reduced in all synaptosomal fractions following methamphetamine administration, indicating that VMAT2 was redistributed from vesicles to a non-synaptosomal site [30].

The primary cause of METH's acute effects is its interaction with the dopamine transporter (DAT) and VMAT-2, which control dopamine release [4]. Both people and animals' DA systems can be impaired by prolonged methamphetamine use. Consequently, it makes sense to assume that part of the cognitive deficiencies may be caused by methamphetamine-induced damage to DA systems [7]. This evidence could explain the positive correlation between VMAT-2 with the DA but not with NA

However, METH administration has been shown to have a major impact on noradrenaline regulation, mostly as a result of its neurochemical interactions with the dopaminergic systems. Increased levels of extracellular monoamines, such as NA, can cause dysregulation in the peripheral immune system and central nervous system (CNS), which can result in neurotoxic consequences and changes in behavior [1]. METH causes the release of dopamine (DA) from vesicular pools, which is then accumulated in monoaminergic terminals and released into the synaptic cleft via DAT. DA auto-oxidation occurs in extracellular and intraneuronal regions as a result of increased DA levels [13]. METH acutely stimulates dopamine release and striatal extracellular reactive oxygen species (ROS). Depletion of noradrenaline intensifies this effect, indicating that NA has a neuroprotective function. There is a crucial relationship between NA and METH toxicity, as its depletion enhances the neurochemical and neurotoxic consequences of METH on striatal DA terminals [31]. A biomarker of sympathetic nervous system activity that corresponds with plasma noradrenaline levels, elevated salivary alpha-amylase activity, suggests that METH therapy raises noradrenaline levels [32]. For dopaminergic neurons, this may intensify neurotoxic consequences [31]. These evidences agree with our results that found significantly positive correlation between NA and DA, and elevated of NA level after treated with METH as neuroprotective parameters in central and peripheral nervous tissues (Brain, Spleen and Kidney).

Despite earlier research suggesting that METH-induced DAT deficits represented irreversible terminal degeneration [33], investigations in nonhuman primates and rats have shown significant recovery with prolonged abstinence [34]. Through a mechanism that is dependent on protein kinase C, METH temporarily suppresses the surface expression of DAT [35].

The current investigation showed that after methamphetamine exposure, renal dopamine (DA) and noradrenaline (NA) levels significantly increased. Since the kidney has its own autonomous dopaminergic system, this increase may be due to kidney-intrinsic catecholamine synthesis rather than just sympathetic spillover [36]. Crucially, methamphetamine toxicity is tightly linked to oxidative stress and inflammation, both of which are known to increase this intrinsic route. These mechanisms provide a reasonable explanation for the renal findings seen in this investigation, even though the exact cellular origin of renal catecholamines was not precisely evaluated.

There are two possible explanations for the significant rise in renal VMAT-2 expression seen in this investigation. First, increased cytosolic catecholamines may cause VMAT-2 overexpression as a compensatory reaction. Elevated VMAT-2 expression limits cytosolic accumulation and lessens monoamine-induced oxidative stress by

facilitating vesicular sequestration of monoamines under conditions of elevated dopamine and noradrenaline availability. Non-neuronal tissues subjected to increased monoaminergic or oxidative burden have been shown to exhibit such protective upregulation [37, 38].

Alternatively, dysregulated monoamine processing brought on by methamphetamine poisoning could be the cause of the rise in VMAT-2. Methamphetamine is known to cause aberrant vesicular loading, accelerated monoamine turnover, and improved oxidative metabolism by interfering with vesicular monoamine dynamics and disrupting normal VMAT-2 control. This imbalance may lead to increased generation of reactive oxygen species and inflammatory signals in peripheral organs, including the kidney [39, 40]. In this situation, elevated VMAT-2 expression may indicate persistent cellular stress rather than a successful protective adaptation, especially when combined with indicators of innate immunological activation as TLR-4 [40, 41].

In the current study, dopamine levels in the group treated with the lowest concentration of METH returned to similar levels in the control, while in the group treated with the high concentration levels continued to increase. However, the entire group treated with the over highest concentration of METH died. The DA transporter increases with abstinence could indicate to one of the following: 1) Methamphetamine-induced DA transporter loss reflects temporary adaptive changes (down regulation). 2) The loss reflects DA terminal damage but that terminals can recover. 3) Remaining viable terminals increase synaptic arborization. This suggests that the increase of the DA transporters was not sufficient for complete function recovery [42].

Because acute METH administration reverses DA transport and displaces DA from vesicular storage, it increases extracellular dopamine levels. In rats and nonhuman primates, repeated treatment results in a reduction of DA metabolites and striatal DA concentrations in various regions of the brain [6]. The availability of D2 receptors is significantly lower in METH abusers than in control subjects, and it is linked to metabolic processes in the orbitofrontal cortex in both groups [34]. Research indicates that frequent administration of METH causes dopamine cells to undergo long-term damage [31]. When compared to saline controls, one study found that the dosage regimen resulted in a more than two-fold reduction in striatal dopamine levels [43], It has been shown that pesticides disrupt dopamine transmission in a manner similar to that of methamphetamine [44].

In brain, METH-stimulated dopamine efflux causes extracellular dopamine levels to increase quickly. METH prevents dopamine from being stored in synaptic vesicles [1]. These facts could be the reason why there was highest levels of DA found when use the highest METH concentration in our study. The pH gradient necessary for dopamine transport to synaptic vesicles is disrupted by METH's structural similarity to dopamine, which promotes competition by VMAT2 [17]. METH also inhibits further dopamine vesicular packing by activating the reserpine-binding region on VMAT2 [18]. Taken together, these evidences might explain the significantly positive correlation between the level of DA and VMAT-2.

Furthermore, the current study found a significantly increase in TLR4 in the highest concentration of METH, which agrees with other studies that found expression in microglial cells and in individuals that have a history of METH use, suggesting that TLR4 activation is linked to METH-induced neuroinflammation [24, 45]. METH damages phagocytic cells, including microglia, and disrupts immunological cellular and molecular functions [46]. Microglia have been linked to brain tissue degradation and neurotoxicity by METH [47]. According to new research, METH affects the central nervous system's innate immune cells, or microglia, which causes the production of proinflammatory mediators that intensify the alterations in neuronal activity caused on by METH. The processes by which METH causes neuroinflammation are still unknown, though. TLR4 signaling mediates METH-induced microglial activation, and TLR4-IL6 signaling in the ventral tegmental area (VTA) partially mediates its neuroinflammatory effects. This route contributes to METH-induced conditioned place preference (CPP), a behavioral indicator of drug reward, by increasing dopamine levels in the nucleus accumbens (NAc) [25].

The investigation of TLR4's role in drug reward began with studies on morphine, the most significant protein molecule involved in both non-specific and specific immune responses is thought to be TLR 4, which was shown to activate microglia as well as release cytokines that promote inflammation [48]. These effects were mediated via the non-stereo-selective innate immune receptor TLR4, not via classical opioid receptors [49]. The way TLR4 signaling affects dopaminergic function may be explained if the treatment of METH causes the release of proinflammatory cytokines. These cytokines may enhance neuroexcitatory effects by increasing glutamate transmission, increasing spontaneous neurotransmitter release, improving receptor conductivity, and upregulating receptor expression [50].

The 10 mg/g methamphetamine group's total death highlights a hazardous threshold at which adaptive monoaminergic and inflammatory responses are overpowered [51]. The convergence of monoaminergic stress and inflammatory injury in peripheral organs like the kidney is suggested by the marked dysregulation of vesicular monoamine handling, which is demonstrated by significant changes in VMAT-2 expression, along with strong activation of innate immune signaling, as indicated by elevated TLR-4 [52, 53]. These peripheral disruptions probably contribute to systemic failure during withdrawal when combined with the known central effects of methamphetamine, such as severe catecholamine depletion, oxidative stress, and autonomic instability [6, 7]. Therefore, rather than isolated organ failure, the best explanation for mortality at a high dose is synergistic central and peripheral toxicity [53]

CONCLUSION

These results may prove that low-dose of METH addiction can be treated simply by stopping METH use while moderate-dose could causes more severe damage that may require treatment but high-dose can cause withdrawal symptoms that can lead to death. On other hand, the effects of METH addiction were not limited to the central nervous system, but also affected the peripheral nervous system in both lymphatic and non-lymphatic organs.

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AUTHORS CONTRIBUTIONS

Haneen Abdulsalam (HA) contributed to the study conception and design, performed the experimental work, and drafted the initial manuscript. Jamela Jouda (JJ)participated in data analysis, interpretation of results, and critical revision of the manuscript. Hawraa Hasan Jasim (HJ) contributed to methodology development, supervision of the research work, and final editing and approval of the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest stated by the authors.

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