

Post-Exposure Circulating Monoamine Alterations and Cerebral Cortex and Striatal Neuropathology Following Repeated Mkpurummiri Exposure in Female and Male Wistar Rats

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ABSTRACT

Illicit psychoactive street preparations represent a new neurotoxicological issue since their unclear and varied composition may result in consequences that cannot be predicted on the basis of their supposed drug identification. This study examined neurochemical and neuropathological modifications after repeated administration of mkpurummiri, a chemically characterised illicit street concoction from Southeast Nigeria. Male and female Wistar rats received mkpurummiri intraperitoneally once daily for 28 days at doses of 1, 2 or 5 mg/kg; control rats received vehicle. Histopathological changes and lesions were studied semiquantitatively in the cerebral cortex and striatum and circulating dopamine (DA), noradrenaline (NA) and serotonin (5-HT) were measured 24 h after the final injection. Mkpurummiri significantly affected all three circulating monoamines in both sexes ($p < 0.001$). Decreases in DA and NA and elevations in 5-HT were seen in an exposure-related manner. DA was decreased by 75.3% and 78.3%, NA by 47.1% and 47.2%, 5-HT was elevated by 446.6% and 589.1% in females and males, respectively, at 5 mg/kg. There was significant exposure $\times$ sex interactions for DA ($p = 0.026$) and 5-HT ($p = 0.013$). Histopathology demonstrated increasing neuronal degeneration and necrosis, reactive cellular alterations and vascular/neurovascular abnormalities in cerebral cortex and striatum. These findings provide complementary neurochemical and morphological evidence of neurotoxicity related with mkpurummiri and show biological changes observable 24 h after final exposure.

Keywords: Mkpurummiri; new psychoactive substances; circulating monoamines; neurotoxicity; cerebral cortex; striatum.

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INTRODUCTION

New psychoactive substances (NPS) are a fast-changing collection of chemically and pharmacologically varied molecules which continue to pose toxicological surveillance, clinical management and public-health response challenges. These are stimulant, cannabinoid, hallucinogenic, dissociative, sedative and opioid-like substances and the continuous structural modification and emergence of new compounds have made their identification and toxicological characterization complicated [1]. The toxicological problem does not stop at the NPS

individually defined, but includes the illicit street preparations where the uncertainty of composition, variable concentrations and the presence of other psychoactive constituents can make it difficult to predict the biological effects from the product name or the supposed active compound. The toxicological evaluation of such formulations, then, needs the study of the material actually encountered and delivered, rather than extrapolation from an assumed pharmacological identity.

Several kinds of NPS, particularly stimulant-type chemicals, are known to have the nervous system as

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a major target of toxicity. Many of the new psychoactive stimulants work on monoaminergic systems via interaction with dopamine, noradrenaline and serotonin transporters, however the pharmacological profiles and relative activity of the substances differ considerably ([2] Besides acute monoaminergic effects, experimental evidence has linked oxidative stress, mitochondrial dysfunction, cytotoxicity and other cellular abnormalities to the neurotoxic potential of stimulant NPS [3]. Synthetic cathinones are a major class of stimulant NPS, and have been shown experimentally to disrupt monoaminergic function and cause neuronal toxicity, although the extent and nature of these effects vary greatly between individual compounds, doses and exposure conditions [4]. Methamphetamine, however, serves as an essential comparative toxicological frame of reference, given the wealth of data on its effects on monoaminergic neurotransmission and neuronal integrity. Repeated or high-intensity exposure to methamphetamine has been shown to disrupt dopaminergic, noradrenergic and serotonergic systems and to produce neuronal, inflammatory and oxidative abnormalities in experimental models [5]. However, structural similarity or overlapping stimulant effects do not provide evidence of toxicological equivalence between methamphetamine and specific NPS or chemically heterogeneous street formulations. Indeed, even among synthetic cathinones that have pharmacology similar to amphetamine-like stimulants, there have been notable variances observed in their neurotoxic potential [6]. Therefore, the proper use of verified methamphetamine is as a comparator for the interpretation of key neurotoxicological patterns, not as a surrogate for direct characterization of an unknown street preparation.

In Southeast Nigeria, an illicit psychotropic street preparation known locally as mkpurummiri has become generally associated with methamphetamine use. However, our previous GC-MS and FTIR characterisation of a street sample identified locally as mkpurummiri revealed a chemically heterogeneous profile comprising multiple tentatively identified constituents and diverse functional groups and did not establish methamphetamine as the predominant constituent [7]. The term mkpurummiri does not necessarily imply that it is chemically pure methamphetamine and its chemical identity and toxicological consequences cannot be accurately extrapolated from its local designation or suspected link with methamphetamine. This chemical variability presents an essential toxicological question, i.e., whether repeated exposure to the preparation as found on the illicit market leads to observable neurochemical and structural changes in the nervous system. Such effects may be due to individual ingredients, interactions between constituents, or features of the combination itself, and so may not be well anticipated from an assumed single-drug identity.

Neurochemical and neuropathological endpoints provide complementary information to study such toxicity. Dopamine (DA), noradrenaline (NA) and serotonin (5-HT) are major monoaminergic mediators targeted by numerous psychoactive stimulants and stimulant-type NPS. Disruption of monoamine homeostasis is an important component of their pharmacological and toxicological [8]. Structural evaluation of brain tissue can uncover neuronal, reactive and neurovascular abnormalities linked with toxic exposure in parallel. The cerebral cortex and striatum are of special interest, as they are involved in cognitive, behavioural and motor activities and possess a rich monoaminergic circuitry. Critically, circulating monoamine levels and regional brain morphology are separate biological goals and monoamines measured in peripheral blood cannot be expected to reflect neurotransmitter concentrations in the cerebral cortex or striatum [9].

Sex differences in neurotoxic reactions to psychoactive stimulants may be related to sex differences in pharmacokinetics, monoaminergic control, endocrine effects, and brain responses to repeated exposure. Contemporary experimental evidence suggests that methamphetamine-associated dopaminergic effects may change as a function of sex, brain region and neurochemical endpoint, but not uniformly across experimental paradigms [10]. Therefore, it is vital to include both female and male animals when researching a poorly characterized psychoactive street preparation, and to analyse potential sex-related patterns without implying increased vulnerability in either sex.

Although the worry about NPS and chemically unclear illicit psychoactive preparations is growing, experimental data on the neurotoxicological effects of prolonged exposure to mkpurummiri are lacking. The present investigation therefore evaluated the effects of repeated exposure to a chemically characterised mkpurummiri street preparation on the amounts of circulating DA, NA and 5-HT and the histological integrity of the cerebral cortex and striatum in female and male Wistar rats. Animals were administered graded doses for 28 days and circulating monoamines were assessed 24 h after the final administration to define a post-exposure circulating monoamine profile. The study combined circulating monoamine measurements with region-specific semiquantitative neuropathological assessment to characterise the neurochemical and morphological patterns associated with repeated mkpurummiri exposure and whether these responses were dependent on exposure level and sex.

MATERIALS AND METHODS

Mkpurummiri sample and chemical characterization

The illicit psychoactive street preparation locally known as mkpurummiri was obtained from a street source in Owerri, Imo State, South-East Nigeria, in

September 2024. The sample was previously characterized by gas chromatography–mass spectrometry (GC–MS) and Fourier-transform infrared (FTIR) spectroscopy. The analyses demonstrated a chemically heterogeneous profile comprising multiple tentatively identified constituents and diverse functional groups and did not establish methamphetamine as the predominant constituent. The characterized street preparation was used for the present experimental study.

Experimental animals and ethical approval

Female and male Wistar rats aged 8–10 weeks and weighing 90–130 g were used. Animals were acclimatized for two weeks under standard laboratory conditions and provided standard laboratory feed and water ad libitum. All experimental procedures involving animals were conducted in accordance with institutional requirements for the care and use of laboratory animals and the principles of the *Guide for the Care and Use of Laboratory Animals* of the National Research Council. Ethical approval for the study was granted by the Imo State University Research and Innovations Committee (IMSU-DRIS), Imo State University, Owerri, Nigeria (Approval No. IMSU/IRB/2026/MED-BIOCHEM-014).

Experimental design and mkpurummiri exposure

Twenty-four Wistar rats were included in the study and separated by sex into female and male experimental sets. Within each sex, animals were assigned to four groups ($n = 3/\text{group/sex}$): normal control (NC), 1 mg/kg mkpurummiri (1M), 2 mg/kg mkpurummiri (2M), and 5 mg/kg mkpurummiri (5M). Animals in the exposure groups received mkpurummiri at 1, 2, or 5 mg/kg body weight by intraperitoneal administration once daily for 28 consecutive days. Normal-control animals received normal saline by the same route and schedule.

Blood collection and determination of circulating monoamines

Blood samples were collected 24 h after the final mkpurummiri administration, and serum was separated for determination of circulating dopamine (DA), noradrenaline (NA), and serotonin (5-HT). The 24-h post-exposure interval was deliberately selected to assess the monoamine profile beyond the immediate acute response to mkpurummiri administration. This sampling window was intended to provide an experimental approximation of the post-use period between episodes of exposure and to assess biological alterations that remained detectable beyond the immediate exposure period.

Circulating monoamine concentrations were determined using rat-specific enzyme-linked immunosorbent assay (ELISA) kits obtained from Wuhan Fine Biotech Co., Ltd., China, according to the manufacturer's instructions. Analyte concentrations were calculated from the corresponding standard calibration curves and expressed as pg/mL.

Because DA, NA, and 5-HT were determined in serum rather than directly in brain tissue, the measured concentrations are reported throughout this study as circulating monoamine concentrations. They were not interpreted as direct measures of neurotransmitter concentrations within the cerebral cortex or striatum.

Brain tissue processing and histopathological examination

Following completion of the experimental period, brain tissues were collected for histopathological examination. Tissue specimens containing the cerebral cortex and striatum were fixed in 10% buffered formalin, processed routinely, and embedded in paraffin wax. Sections of 5 μm thickness were cut, mounted on glass slides, and stained with haematoxylin and eosin (H&E).

The stained sections were examined under a light microscope by a pathologist, and representative photomicrographs were captured using a charge-coupled device camera at $\times 400$ magnification. Histopathological evaluation focused separately on the cerebral cortex and striatum to preserve the region-specific morphological features observed following mkpurummiri exposure.

Semiquantitative neuropathological assessment

Neuropathological alterations were evaluated using a semiquantitative severity-grading approach adapted to the morphological features observed in each brain region. The principles of reproducible ordinal histopathological scoring were followed, with severity assigned according to the extent of the observed lesion rather than treating the scores as continuous measurements.

For the cerebral cortex, five lesion domains were evaluated: dark/degenerating neurons, neuronal necrosis, abnormal cellular changes, vascular alterations, and reactive/glial changes.

For the striatum, the evaluated domains were dark/degenerating neurons, neuronal necrosis, neuronal cell loss, reactive/glial changes, and vascular/neurovascular abnormalities.

Each domain was graded on a 0–4 ordinal scale:

- 0 = absent;
- 1 = minimal;
- 2 = mild;
- 3 = moderate;
- 4 = severe.

The five domain scores were summed to obtain a composite neuropathological severity score ranging from 0 to 20 for each brain region. Cortex and striatum were scored independently because the defining pathological features were not identical between the two regions.

Statistical analysis

Statistical analyses were performed using JASP statistical software. Circulating dopamine (DA), noradrenaline (NA), and serotonin (5-HT) concentrations are presented as mean $\pm$ standard deviation (SD), with $n = 3$ animals per group per sex. Within each sex, differences among the normal control and mkpurummiri-exposed groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple-comparison test where appropriate. The effects of exposure level, sex, and exposure $\times$ sex interaction on each circulating monoamine were further evaluated using two-way ANOVA. Statistical significance was accepted at $p < 0.05$.

Neuropathological alterations in the cerebral cortex and striatum were assessed semiquantitatively in each animal using the 0–4 ordinal severity-grading system described above. For each lesion domain, the scores obtained from the three animals within each group and sex were averaged to provide the group-level average severity score presented in Tables 2 and 3. The average lesion-domain scores were summed to obtain the corresponding composite neuropathological severity score for each group and sex. Because these histopathological scores were used primarily for

descriptive semiquantitative characterization of lesion severity and the number of animals per group was small ($n = 3$), no inferential statistical comparisons were performed on the neuropathological severity scores.

RESULTS

Effects of mkpurummiri exposure on circulating monoamine concentrations

Repeated mkpurummiri exposure significantly altered circulating monoamine concentrations in female and male rats (Table 1). DA and NA concentrations decreased with increasing exposure, whereas 5-HT increased. Within-sex analysis showed significant exposure effects for all three monoamines in females (DA: $F(3,8) = 96.42$; NA: $F(3,8) = 224.64$; 5-HT: $F(3,8) = 121.08$; all $p < 0.001$) and males (DA: $F(3,8) = 154.33$; NA: $F(3,8) = 111.86$; 5-HT: $F(3,8) = 128.01$; all $p < 0.001$).

Two-way ANOVA confirmed significant effects of exposure level on DA, NA and 5-HT (all $p < 0.001$). Sex had a significant main effect on NA ($p < 0.001$), but not DA ($p = 0.166$) or 5-HT ($p = 0.857$). Exposure $\times$ sex interactions were significant for DA ($p = 0.026$) and 5-HT ($p = 0.013$), but not NA ($p = 0.102$).

Table 1. Effects of repeated mkpurummiri exposure on circulating monoamine concentrations in female and male Wistar rats

	Dopamine (pg/ml)		Noradrenaline (pg/ml)		Serotonin (pg/ml)	
	Female	Male	Female	Male	Female	Male
Normal Control	8092.01 $\pm$ 175.43 ^a	7846.87 $\pm$ 216.46 ^a	142.31 $\pm$ 3.12 ^a	160.20 $\pm$ 5.39 ^a	78.92 $\pm$ 3.44 ^a	72.63 $\pm$ 4.25 ^a
1mg/kg mkpurummiri	4021.71 $\pm$ 932.42 ^b	4363.06 $\pm$ 315.02 ^b	94.43 $\pm$ 4.12 ^b	109.90 $\pm$ 4.66 ^b	120.04 $\pm$ 11.19 ^a	100.55 $\pm$ 1.89 ^a
2mg/kg mkpurummiri	2744.45 $\pm$ 135.55 ^c	3937.43 $\pm$ 594.54 ^b	82.55 $\pm$ 1.72 ^c	87.06 $\pm$ 8.57 ^c	331.07 $\pm$ 26.36 ^b	279.11 $\pm$ 21.73 ^b
5mg/kg mkpurummiri	1997.72 $\pm$ 15.23 ^c	1706.46 $\pm$ 39.62 ^c	75.31 $\pm$ 4.35 ^c	84.53 $\pm$ 2.81 ^c	431.35 $\pm$ 44.55 ^c	500.61 $\pm$ 56.20 ^c

Data are presented as mean $\pm$ SD ($n = 3$ animals/group/sex). Female and male values are presented side-by-side for each parameter. DA, dopamine; NA, noradrenaline; 5-HT, serotonin; NC, normal control; 1M, 1mg/kg mkpurummiri; 2M, 2mg/kg mkpurummiri; 5M, 5mg/kg mkpurummiri. Within each sex and monoamine parameter, means sharing at least one common superscript letter are not significantly different, whereas means with no common superscript letters differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's post hoc multiple-comparison test).

Mkpurummiri-induced neuropathological alterations in the cerebral cortex

Repeated mkpurummiri exposure produced exposure-related histopathological alterations in the cerebral cortex of female and male Wistar rats (Table 2; Figure 1). The normal-control groups showed preserved cortical architecture, whereas exposed animals exhibited progressively greater dark/degenerating neurons, neuronal necrosis, abnormal cellular changes, vascular alterations, and reactive/glial changes. These abnormalities became more pronounced with increasing

exposure, with the greatest overall cortical lesion severity observed at 5 mg/kg in both sexes.

Semiquantitative assessment supported the exposure-related progression of cortical injury. Composite neuropathological severity increased across the mkpurummiri-exposed groups relative to the corresponding controls. The principal morphological changes and their graded severity are presented in Table 2, while representative cortical photomicrographs are shown in Figure 1.

Table 2. Semiquantitative neuropathological severity of the cerebral cortex following repeated mkpurummiri exposure in female and male Wistar rats

Histopathological feature	Normal Control		1mg/kg		2mg/kg		5mg/kg	
	Female	Male	Female	Male	Female	Male	Female	Male
Degenerating neurons	0	0	1	2	3	3	4	4
Necrotic neurons	0	0	1	1	2	2	4	3
Abnormal cellular changes	0	0	1	2	3	3	3	4
Vascular alterations	0	0	1	1	2	2	3	3
Reactive/glial changes	0	0	1	1	2	2	3	3
Total score (/20)	0	0	5	7	12	12	17	17

Neuropathological alterations were graded independently in each animal using a 0–4 ordinal scale: 0, absent; 1, minimal; 2, mild; 3, moderate; and 4, severe. Values shown represent the average severity score for three animals per group per sex ($n = 3$). Composite neuropathological severity scores were obtained by summing the average scores for dark/degenerating neurons, neuronal necrosis, abnormal cellular changes, vascular alterations, and reactive/glial changes, giving a maximum possible composite score of 20.

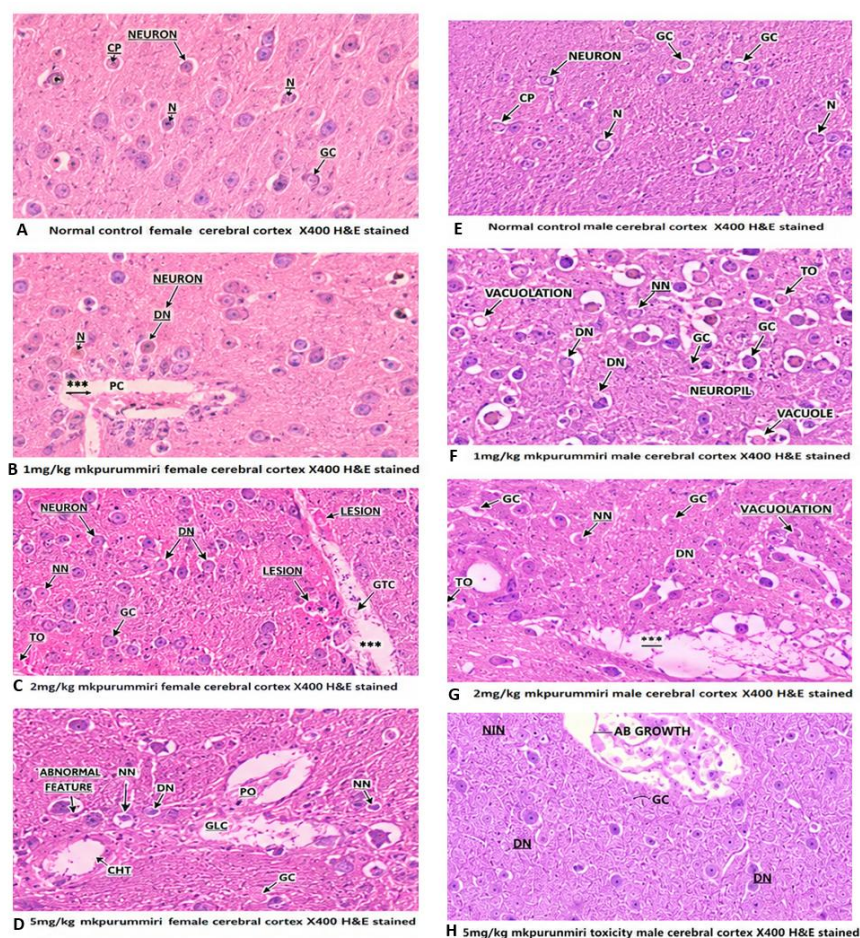


Figure 1. Cerebral cortex: H&E-stained sections (×400)

Figure 1. Representative photomicrographs of the cerebral cortex of female and male Wistar rats following repeated mkpurummiri exposure for 28 days.

H&E, ×400. Normal-control sections demonstrate preserved cortical architecture, whereas exposed groups show increasing dark/degenerating neurons, neuronal necrosis, abnormal cellular changes, vascular alterations, and reactive/glial changes with increasing exposure

Mkpurummiri-induced neuropathological alterations in the striatum

Repeated mkpurummiri exposure produced exposure-related neuropathological alterations in the striatum of female and male Wistar rats (Table 3; Figure

2). Striatal architecture was preserved in the normal-control groups, whereas exposed animals showed increasing dark/degenerating neurons, neuronal necrosis, neuronal cell loss, reactive/glial changes, and vascular/neurovascular abnormalities. The extent and

severity of these alterations generally increased with exposure level, with the most pronounced striatal abnormalities observed at 5 mg/kg in both sexes.

Semiquantitative assessment supported the progressive increase in striatal neuropathological

severity across the mkpurummiri-exposed groups relative to the corresponding controls. The individual lesion domains and composite severity scores are presented in Table 3, while representative photomicrographs demonstrate the associated morphological changes in Figure 2.

Table 3. Semiquantitative neuropathological severity of the striatum following repeated mkpurummiri exposure in female and male Wistar rats

Histopathological feature	Normal Control		1mg/kg		2mg/kg		5mg/kg	
	Female	Male	Female	Male	Female	Male	Female	Male
Degenerating neurons	0	0	1	2	3	3	4	4
Necrotic neurons	0	0	1	1	2	2	3	3
Neuronal cell loss	0	0	1	1	2	2	3	3
Reactive/glial changes	0	0	1	1	2	2	3	3
Vascular/neurovascular alterations	0	0	2	2	3	3	4	4
Total score (/20)	0	0	6	7	12	12	17	17

Neuropathological alterations were graded independently in each animal using a 0–4 ordinal scale: 0, absent; 1, minimal; 2, mild; 3, moderate; and 4, severe. Values shown represent the average severity score for three animals per group per sex (n = 3). Composite neuropathological severity scores were obtained by summing the average scores for dark/degenerating neurons, neuronal necrosis, neuronal cell loss, reactive/glial changes, and vascular/neurovascular abnormalities, giving a maximum possible composite score of 20.

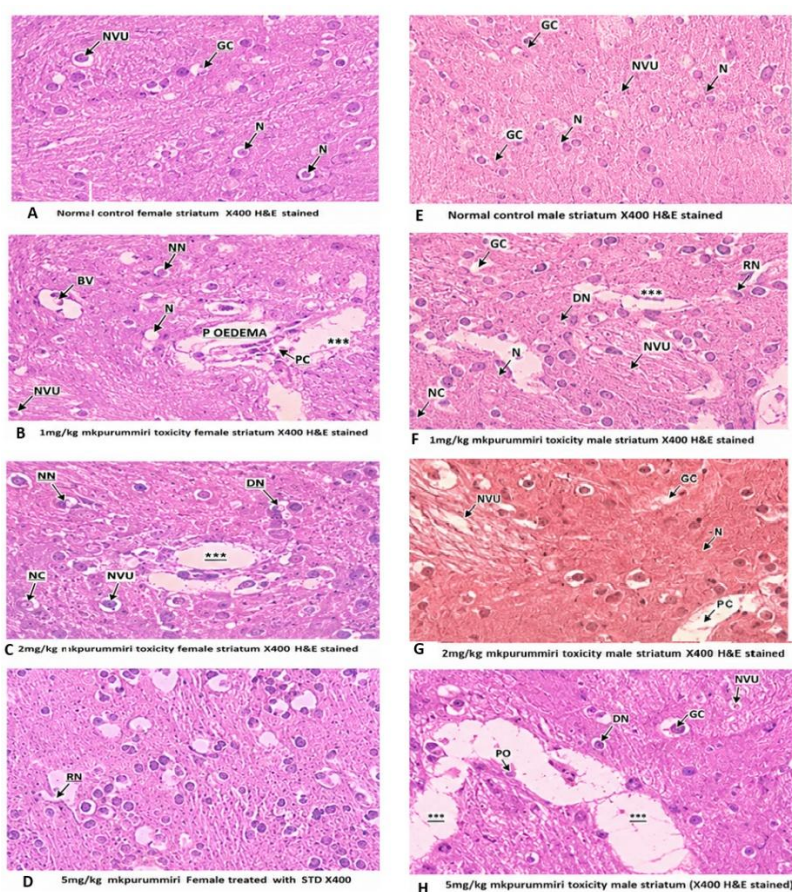


Figure 2. Cerebral striatum: H&E-stained sections (x400)

Figure 2. Representative photomicrographs of the striatum of female and male Wistar rats following repeated mkpurummiri exposure for 28 days.

H&E, x400. Normal-control sections demonstrate preserved striatal architecture, whereas Mkpurummiri-exposed groups show progressively greater dark/degenerating neurons, neuronal necrosis, neuronal cell loss, reactive/glial changes, and vascular/neurovascular abnormalities with increasing exposure.

Exposure-, sex-, and region-related patterns of mkpurummiri-associated neurotoxicity

Integration of the neurochemical and neuropathological findings showed a consistent exposure-related pattern of mkpurummiri-associated neurotoxicity in both sexes. Increasing exposure was accompanied by reductions in circulating DA and NA, an increase in circulating 5-HT, and progressively greater neuropathological severity in the cerebral cortex and striatum. The most pronounced neurochemical alterations and morphological abnormalities generally occurred at the highest exposure level.

Sex-related effects differed according to the endpoint examined. Two-way ANOVA demonstrated a significant main effect of sex on circulating NA ($p < 0.001$), whereas the main effect of sex was not significant for DA ($p = 0.166$) or 5-HT ($p = 0.857$). Significant exposure $\times$ sex interactions were observed for DA ($p = 0.026$) and 5-HT ($p = 0.013$), indicating that the exposure-related patterns of these monoamines differed between females and males. The exposure $\times$ sex interaction for NA was not significant ($p = 0.102$).

Neuropathological alterations occurred in both brain regions but were characterized by partly distinct morphological patterns. The cerebral cortex showed dark/degenerating neurons, neuronal necrosis, abnormal cellular changes, vascular alterations, and reactive/glia changes, whereas the striatum was characterized by dark/degenerating neurons, neuronal necrosis, neuronal cell loss, reactive/glia changes, and vascular/neurovascular abnormalities. Thus, repeated mkpurummiri exposure was associated with a convergent pattern of increasing neuropathological severity across the two regions while retaining region-specific morphological features.

DISCUSSION

The present work indicates that repeated exposure to the unlawful psychoactive street concoction mkpurummiri induces a coordinated pattern of neurochemical and neuropathological changes in female and male Wistar rats. Critically, circulating monoamines were measured 24 h after the final injection and not during the acute period after exposure. At this defined post-exposure period, circulating dopamine (DA) and noradrenaline (NA) were decreased, while serotonin (5-HT) was elevated, with substantial exposure effects for all three monoamines. These changes were paralleled by increasing neuropathological abnormalities in the cerebral cortex and striatum. Therefore, the data provide evidence of persistent quantifiable monoaminergic abnormalities beyond the acute exposure period and of coexisting anatomical evidence of brain injury after recurrent mkpurummiri treatment.

These results must be considered in the light of the type of the preparation delivered. Mkpurummiri is widely believed to be a methamphetamine preparation in

Southeast Nigeria. However, our previous characterisation using GC-MS and FTIR revealed that the street preparation was chemically heterogeneous with multiple tentatively identified constituents and a variety of functional groups, and did not show methamphetamine to be the predominant constituent [11]. Therefore, the neurotoxicological findings presented here are due to the mkpurummiri formulation as it was delivered and not only to methamphetamine. This distinction is especially relevant to the toxicology of new psychoactive substances (NPS) and emerging illicit stimulant preparations, for which chemical modifications, uncertain composition and differences in monoamine-transporter activity can produce substantially different pharmacological and toxicological profiles [12]. Thus methamphetamine provides a valuable comparative paradigm for analysing selected findings, but should not be taken as a substitute chemical identity for mkpurummiri. The 24-h sampling interval was chosen to assess the post-exposure condition following the immediate acute response to injection. The design was aimed at providing an experimental environment for the interval that might elapse between episodes of illicit psychoactive substance use, during which the immediate subjective or pharmacological effects of exposure may have waned, but biological changes might still be evident. The substantial changes in circulating DA, NA and 5-HT at this time point are so remarkable. They show that, 24 h after the last exposure, the monoamine homeostasis has not restored to the pattern found in control mice. However, just one post-exposure time point was tested in the current investigation, which cannot determine when these changes occurred, the course of these changes throughout the previous 24 h, or how long these changes persisted subsequently.

Repeated mkpurummiri exposure results in a marked disruption of monoaminergic control, as reflected by reduced levels of circulating DA and NA. Monoamine systems are important pharmacological targets of stimulant-type NPS such as synthetic cathinones, many of which alter dopamine, noradrenaline and serotonin signalling by their effects at monoamine transporters [13]. Importantly, different stimulant NPS have different selectivity for transporters, potency and whether they act primarily as transporter substrates/releasers or reuptake inhibitors. Recent research continues to show how even slight structural variations between synthetic cathinones can considerably vary their activities at DAT, NET and SERT and thus their pharmacological and toxicological profiles [14]. These findings thus support disruption of monoamine modulation following mkpurummiri exposure but do not identify which chemical or pharmacological activity created the observed pattern.

Methamphetamine provides meaningful comparative evidence for the reduction in DA. Repeated or high intensity methamphetamine exposure has been

linked to dysregulation in dopamine synthesis, transport and metabolism and resultant dopaminergic dysfunction [15]. Experimental investigations suggest that dopaminergic responses to methamphetamine may differ as a function of pattern of exposure, sex and brain area. However, the present findings of DA and NA were from serum and not brain tissue. This means that lower circulating concentrations at 24 h cannot be used as a direct means of inferring depletion of dopamine or noradrenaline in the cerebral cortex or striatum.

Unlike DA and NA, mkpurummiri exposure enhanced circulation 5-HT levels. This differential reaction suggests that the three monoaminergic systems were not uniformly altered by the preparation. This discrepancy is consistent with the varied pharmacology of stimulant NPS. Synthetic cathinones and comparable psychoactive stimulants have a wide range of relative activity at DAT, NET and SERT, with certain compounds having proportionally stronger serotonergic effects than others [16]. Thus, the increase of circulating 5-HT at 24 h may reflect differential post-exposure modulation of serotonergic pathways. However, its biological origin cannot be determined from the current peripheral test and higher serum 5-HT should not be interpreted as enhanced serotonergic neurotransmission within the brain.

These neurochemical changes after exposure were followed by significant anatomical signs of brain damage. In the cerebral cortex, mkpurummiri exposure was associated with dark/degenerating neurones, neuronal necrosis, aberrant cellular changes, vascular abnormalities and reactive/glia changes. In the striatum, we found dark/degenerating neurones, neuronal necrosis, neuronal cell loss, reactive/glia alterations, and vascular/neurovascular abnormalities. The increasing average lesion-severity scores across exposure levels, coupled with the corresponding histopathological findings, indicate that the effects of repeated mkpurummiri administration extended beyond circulating biochemical changes to structural injury in key brain regions both anatomically and functionally.

Neuronal degeneration and necrosis found in the present investigation parallels the larger experimental literature on stimulant-associated neurotoxicity. NeuroToxicology released a systematic review by [17], that reported evidence of methamphetamine-associated cell death in rodent brain regions including the frontal cortex and striatum. Current evidence suggests that treatment with psychostimulants induces brain injury via oxidative stress, mitochondrial dysfunction, neuroinflammation, excitotoxic processes and cell-death pathways [18]. Similarly, neuronal cytotoxicity, mitochondrial dysfunction, oxidative injury and abnormalities in monoaminergic signalling are representative of neurotoxicological evidence for NPS, especially synthetic cathinones [19]. These processes give biologically plausible context for the cortical and

striatal abnormalities found during mkpurummiri exposure. However, they were not explicitly quantified in the present investigation, and should be consequently treated as literature-supported mechanistic possibilities and not empirically confirmed mechanisms. The striatum is particularly important in stimulant neurotoxicity because of its heavy dopaminergic innervation and its role in motor, motivational and reward-related activities. The neuronal cell loss, degeneration and necrosis identified in this location give consequently strong morphological evidence of mkpurummiri-associated damage. Experimentally, methamphetamine exposure has been related with striatal dopaminergic dysfunction and neuronal damage [20]. However, the co-occurrence of lower circulating DA and striatal disease in the present investigation does not imply a clear causal link between these end-points. Peripheral DA concentration and striatal dopaminergic neurotransmission are biologically independent. Direct measurement of regional brain monoamines would be required to determine their relationship.

The cerebral cortex was also significantly impacted, indicating that mkpurummiri-associated neuropathology was not limited to the striatum. Cortical neuronal degeneration and necrosis were accompanied by aberrant cellular, reactive/glia and vascular changes. Neurotoxicity linked with psychostimulants can include both cortical and subcortical systems, with the pattern and extent of damage depending on the substance, the exposure intensity and the experimental paradigm [21]. Thus, the participation of both cerebral cortex and striatum in the present investigation shows a more extensive regional pattern of neuropathological impairment following repeated mkpurummiri exposure.

The vascular and neurovascular anomalies encountered in both locations deserve special attention. Neurovascular dysfunction may mediate neuronal damage via changes in endothelial integrity, cerebral perfusion and blood-brain barrier homeostasis. Exposure to methamphetamine has been linked to structural and functional abnormalities of the blood-brain barrier, with oxidative stress and neuroinflammation being suggested as contributory factors [22]. Thus, the vascular abnormalities detected histologically in the present investigation are biologically important, but should not be taken as direct evidence of blood-brain barrier disruption because barrier permeability, endothelial markers and cerebral blood flow were not examined. Sex-dependent changes were seen in some neurochemical responses. Two-way analysis showed a significant main influence of sex on circulating NA. The main effects of sex on DA and 5-HT were not significant. Conversely, we found significant exposure $\times$ sex interactions for DA and 5-HT, suggesting that their reactions over increasing mkpurummiri exposure differed between females and males. Sex-dependent changes in dopaminergic modulation have also been observed in rats following methamphetamine

self-administration [23]. These data highlight the need to test both sexes in the evaluation of poorly characterised psychoactive preparations and caution against the assumption that exposure escalation causes similar neurochemical effects in females and males.

One strength of the present investigation is the inclusion of a purposefully specified 24-h post-exposure neurochemical assessment with independently assessed neuropathology in two brain areas and both sexes. The experimental design shows evidence of biological anomalies that can be identified beyond the acute exposure period, and not just characterising the initial reaction to mkpurummiri administration. This has translational importance to repeated illicit psychoactive substance use, as the durations between bouts of exposure represent an element of the real-world pattern of usage. However, the model should not be construed as mimicking the complexity of real mkpurummiri use, where dose, frequency, method of administration, co-exposures, nutritional state and other individual characteristics may vary greatly.

So there are certain constraints to remember. First, circulating DA, NA and 5-HT were assessed at one single 24 h post-exposure time point. The results confirm that changes were apparent at that time but cannot tell when they began, how long they lasted, or subsequent recovery. Second, serum monoamine concentrations are not surrogates of neurotransmitter concentrations in the cerebral cortex or striatum. Third, oxidative stress, neuroinflammatory, mitochondrial, apoptotic and blood–brain barrier indicators were not assessed; therefore, the reported histopathological injury cannot be directly linked to these pathways. Fourth, the limited number of animals per group demands cautious interpretation especially sex-related comparisons. Finally, since mkpurummiri is chemically diverse, the study cannot identify whether the observed effects resulted from a particular constituent, additive activities among elements, synergistic interactions or other aspects of the street concoction.

Taken together, the results extend the toxicological characterisation of mkpurummiri beyond the chemical variability described before. Repeated exposure resulted in significant changes in circulating monoamine levels that persisted for 24 h following the last exposure and exposure-related neuropathological damage in the cerebral cortex and striatum of male and female rats. These results, in the wider context of NPS and new illicit psychoactive preparations, highlight the difficulty of forecasting toxicity based on an assumed street-drug identity and promote direct examination of chemically characterised street preparations. Future studies with multiple time points after exposure, direct measurement of regional brain monoamines and molecular markers of oxidative stress, neuroinflammation, mitochondrial dysfunction, cell death and neurovascular integrity may help define the

temporal course and mechanisms of mkpurummiri-induced neurotoxicity.

CONCLUSION

Repeated exposure to the chemically heterogeneous street preparation mkpurummiri induced exposure-related neurochemical and neuropathological changes in female and male Wistar rats. Twenty-four hours after the last injection, circulating dopamine and noradrenaline were reduced and serotonin was elevated, indicating that abnormalities in monoamine homeostasis were still evident beyond the acute exposure period. These changes were associated with increased neuropathological abnormalities in cerebral cortex and striatum, including neuronal degeneration and necrosis, reactive cellular modifications, and vascular or neurovascular alterations.

The defined 24-h post-exposure assessment offers experimental insight into biological abnormalities that may persist during the period between bouts of illicit psychoactive substance use, but does not mimic the complexity of human exposure. Because mkpurummiri is chemically diverse and monoamines were tested in serum rather than brain tissue, the effects cannot be attributed only to methamphetamine or taken as direct proof of regional brain neurotransmitter depletion. To summarise, these findings provide complementary neurochemical and morphological evidence of mkpurummiri associated neurotoxicity and justify further investigation with multiple post-exposure time points, regional brain neurochemistry and molecular markers to clarify the mechanisms and chemical contributors underlying these effects.

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