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From clinical observation to experimental validation: Investigating the neurotoxic impact of dimethylacetamide

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ABSTRACT

Dimethylacetamide (DMAC) is a polar organic solvent widely used in the production of synthetic fibers and other industrial applications. Its hepatotoxic potential has been well documented in laboratory animals and occupationally exposed workers, establishing DMAC as a recognized industrial hazard. However, evidence regarding its neurological effects remains scarce. We report a clinical case of accidental DMAC inhalation associated with diffuse cortical hyperintensity on brain magnetic resonance imaging, raising concern for direct neurotoxicity. To address this knowledge gap, we investigated the neurotoxic potential of DMAC in a controlled rat model experiment. Animals subjected to repeated intraperitoneal DMAC administration exhibited cortical and subcortical histopathological alterations consistent with neurotoxicity, accompanied by significant hepatic and renal injury. These findings provide the first experimental evidence that DMAC toxicity extends beyond hepatotoxicity to involve the central nervous system and kidneys, highlighting its potential for multi-organ toxicity and reinforcing concerns regarding occupational exposure risks.

1. Introduction

DMAC (N,N-dimethylacetamide; DMAC, CAS no. 127–19–5) is a widely used solvent, particularly for polymer solubilization in the fiber and textile industries (Kennedy, 2012). Due to its extensive industrial applications, particularly in synthetic fiber production, occupational exposure to DMAC has become a significant health concern. DMAC is therefore not only an industrial solvent, but also a chemical that needs to be carefully evaluated from an occupational health perspective (Weiss et al., 1962). From an occupational health perspective, regulatory agencies have established various exposure limits to reduce the risks associated with DMAC. The National Institute for Occupational Safety and Health (NIOSH) recommends an 8-hour time-weighted average (TWA) exposure limit of 10 ppm for DMAC and a short-term exposure limit (STEL) of 20 ppm (U.S. Department of Health and Human Services, 1994). However, even with these values, numerous cases of occupational poisoning have been reported in conditions involving inadequate ventilation or prolonged exposure; toxic effects can occur even when environmental concentrations approach or intermittently exceed the recommended limits.

Currently, the neurotoxicity of DMAC in humans is a complex issue primarily influenced by occupational exposure levels and individual susceptibility. DMAC is generally considered to have low toxicity, but there are indications of potential neurotoxic effects under high exposure conditions. Toxicological investigations in both humans and animals have reported poisoning, hepatotoxicity, fatty infiltration, and focal necrosis following high-dose exposure (Jiang et al., 2024; Liu et al., 2016; Palmen et al., 1993; Weiss et al., 1962). Consistent with these

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findings, liver injury associated with DMAC exposure has also been documented in textile workers (Jung et al., 2007; Nomiyama et al., 2025). An extensive retrospective cohort study of 2795 workers from four European factories further confirmed the correlation between occupational DMAC exposure and hepatotoxic outcomes (Antoniou et al., 2021). Up-to-date findings from animal experiments and epidemiologic studies on worker health should be corroborated.

Particularly, in a recent incident in China, six workers showed symptoms of poisoning after being exposed to DMAC during maintenance activities at a spandex factory (Jiang et al., 2024). This incident, and more recently, highlights the risks associated with occupational exposure when work-safety protocols are not strictly followed. While these events reveal the multidimensional effects of exposure, they also suggest that neurological findings in particular are still poorly understood.

Despite the growing body of evidence concerning its hepatotoxic effects, the potential neurotoxic consequences of exposure to DMAC remain largely unexplored. In this study, we evaluate the neurological impact of intraperitoneally administered DMAC using a controlled repeated-exposure rat model. This study is based on the clinical profile of a patient who exhibited both hepatotoxic manifestations and neurological symptoms. Our study aims to provide a comprehensive toxicological profile of DMAC exposure by combining clinical observations with experimental findings.

2. Case Presentation

A 35-year-old male cleaning worker entered a distillation tank in a textile factory to perform routine cleaning. During this process, he was accidentally exposed to DMAC vapors and subsequently lost consciousness. Around 10 h later, he was found in the tank in a comatose state. He was transferred to the intensive care unit, where he stayed for a week. Laboratory assessments during hospitalisation revealed mildly elevated levels of aspartate aminotransferase (AST), while other biochemical and haematological parameters remained within normal limits.

Upon regaining consciousness, the patient exhibited dysarthria, aphasia, and cortical blindness. No pathological findings were detected during systemic examinations. As the neurological deficits persisted, a brain MR scan was performed one month after exposure. This revealed diffuse cortical hyperintensities on T1, T2, and FLAIR sequences, consistent with cortical laminar necrosis. This was most prominent in the frontal and parietal lobes (see Fig. 1A). Additionally, mild cerebral and cerebellar cortical atrophy was noted. At a later follow-up, the patient continued to exhibit cortical visual impairment, dysarthria, and mild cognitive impairment.

A review of the current literature reveals that data on neurological findings associated with DMAC exposure are somewhat limited. In early studies, apart from isolated cases of hallucinations reported by Weiss et al. in the context of chemotherapy, distinct neurological clinical findings related to DMAC exposure have rarely been described(Weiss et al., 1962). In addition, in the recently reported large-scale occupational exposure cases by Jiang and colleagues, although exposure levels were detailed, there was no mention of neurological symptoms or brain magnetic resonance imaging findings. The isolated cortical involvement observed in the presented case suggests that DMAC may have a potential direct neurotoxic effect on the central nervous system (Jiang et al., 2024). Accordingly, an experimental animal model was designed to elucidate the pathophysiological basis of the observed cortical laminar necrosis in greater detail.

3. Materials and methods

The following experimental protocol was designed to evaluate the neurotoxic and hepatotoxic effects of DMAC exposure.

3.1. DMAC preparation

N,N-dimethylacetamide (DMAC; ≥99.8 % purity; Sigma-Aldrich) was supplied in liquid form and administered intraperitoneally at the indicated doses. Injection volumes were calculated based on body weight.

3.2. Animals

In clinical cases, patients were exposed to DMAC via inhalation; however, in our experimental model, the compound was administered intraperitoneally (i.p.) to ensure controlled and reproducible systemic delivery. The animal study was approved by the Local Experimental Animals Ethics Committee (Approval No: 2022/81) and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Fourteen-week-old male Wistar albino rats were used. Animals were housed four per cage in standard acrylic cages under controlled temperature and humidity conditions with a 12-hour light/dark cycle, and were allowed free access to food and water. Prior to experimentation, the body weights of all rats were recorded.

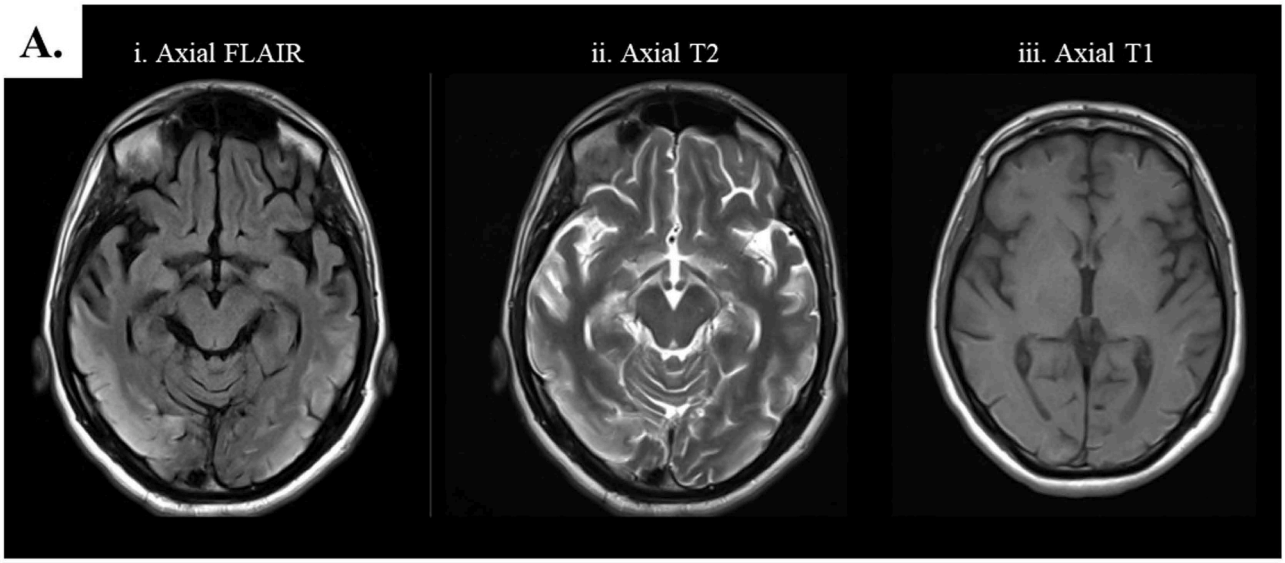
The sample size was determined a priori based on a statistical power analysis conducted for an exploratory in vivo toxicology study. As no prior experimental data or specific effect estimates were available, the analysis was not based on a single predefined dependent variable but relied on conservative assumptions commonly used in hazard-identification studies aimed at detecting sublethal toxicity. Dose selection and group size were guided by published toxicological reference data for DMAC, including reported intraperitoneal LD_{50} values and documented sublethal toxic effects. At a 95 % confidence level, a significance threshold of 0.05, and with 80 % statistical power, the minimum required number of animals per experimental condition was calculated to be three.Rats were randomly assigned to three groups (1000 mg/kg and 2000 mg/kg of DMAC, and isotonic salin for the sham group). The 1000 mg/kg dose group was further divided into subgroups receiving one, two, or three i.p. injections within a 24-hour period to assess the effects of exposure frequency. The 2000 mg/kg dose group received a single i.p. injection only. Although ethical approval permitted longer observation periods, animals were humanely sacrificed 24 h after the final DMAC administration to prioritize animal welfare, allowing MRI, histopathological, and blood analyses while avoiding potential mortality. In addition, sham control animals ($n = 3$) received intraperitoneal injections of isotonic saline following the same injection schedule as the experimental groups. Blood samples were collected after each saline injection. At the endpoint, MRI was performed in one representative animal per group and used as a supportive qualitative assessment, whereas histopathological analyses were conducted in all animals. A schematic representation of the experimental design is shown in Fig. 1B.

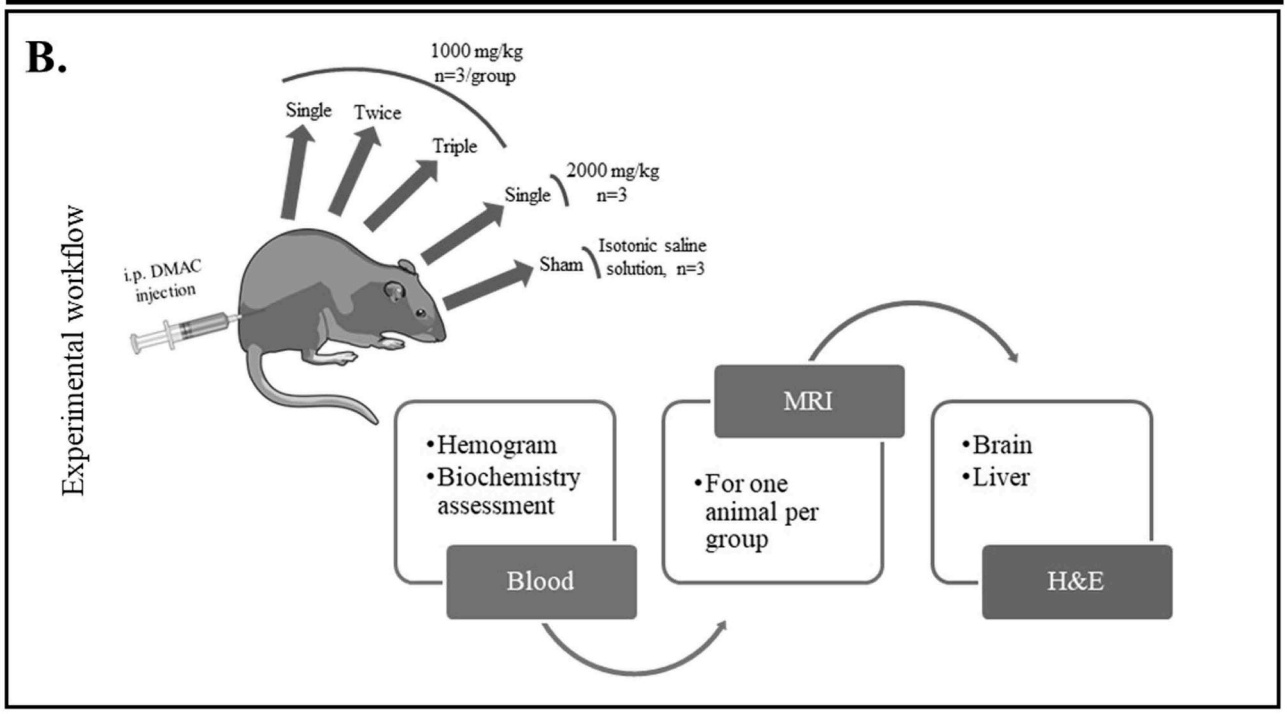
3.3. Biochemical analyses

One milliliter of blood was collected from the jugular vein of each rat both prior to experimental procedures and at sacrifice. Hematological and biochemical parameters were measured using the IDEXX VetTest system (IDEXX, Maine, USA). For hematological analyses, EDTA-anticoagulated whole blood was used. Serum samples were analyzed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Coagulation parameters, including aPTT, PTT, and INR, were measured in sodium-citrate tubes (3.2 %). In this way, coagulation disorders due to possible hepatic damage were also evaluated.

3.4. Magnetic Resonance Imaging (MRI)

To assess the neurotoxic effects, rats exposed to DMAC at doses of 1000 mg/kg and 2000 mg/kg and sham underwent brain MR scanning. Imaging was performed on a 1.5 Tesla MR scanner while the rats were





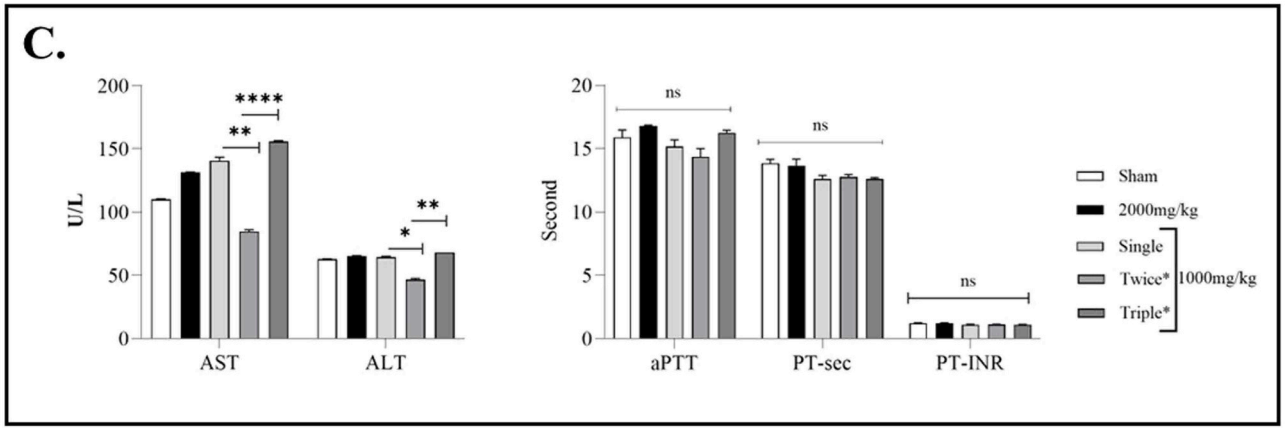


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Fig. 1. A. Brain magnetic resonance imaging (MRI) findings of the clinical case. Bilateral symmetric posterior hyperintense involvement on the axial FLAIR weighted (i.) and T2 weighted brain images (ii.) of cortical and subcortical white matter in the temporal and occipital lobes, with less prominent involvement of the frontal and parietal lobes, is indicative of edema. Bilateral symmetric cortical hyperintensity with sparing of deep structures on axial T1 brain imaging is consistent with pseudolaminar cortical necrosis (iii.). **B.** Experimental workflow. Rats received intraperitoneal (i.p.) administration of DMAC at 1000 mg/kg (single, twice, or triple administration within 24-hour intervals) or a single dose of 2000 mg/kg. Sham controls received isotonic saline (n = 3). Blood samples were collected for hemogram and biochemical analysis. One animal from each group underwent brain MRI examination. Subsequently, all animals were sacrificed, and brain and liver tissues were harvested for histopathological evaluation. **C.** Serum biochemical and coagulation parameters following DMAC administration. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (left panel), and activated partial thromboplastin time (aPTT), prothrombin time (PT), and PT-international normalized ratio (PT-INR) (right panel) are shown for sham controls, single-dose 2000 mg/kg DMAC, and single, twice, and triple administrations of 1000 mg/kg DMAC. Data are presented as mean ± SD. DMAC: dimethylacetamide.

under general anaesthesia. Two-millimetre axial sections were obtained, including T1 axial, T2 axial, T2 CISS, SWI, FLAIR, diffusion-weighted (DWI), and apparent diffusion coefficient (ADC) sequences. The DICOM images were analysed using the OsiriX MD software.

3.5. Histopathology

The liver, kidney, and brain tissues were fixed in 10 % neutral-buffered formalin for 24 h. Tissues were collected immediately after sacrifice and rapidly immersed in ice-cold buffered fixative to minimize postmortem degradation. The samples were then dehydrated in a series of graded alcohols, cleared in xylene, and embedded in paraffin wax. Sections (3–4 μm thick) were cut using a rotary microtome and mounted on positively charged slides. The slides were then deparaffinized at 70°C, rehydrated through a series of descending alcohol and stained with haematoxylin and eosin (H&E) and Masson's trichrome (MT). Histological examinations were performed using light microscopy (Nikon Eclipse 920248, USA) in a blinded manner by two independent investigators unaware of group assignments.

3.6. Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 10). Data are presented as mean ± standard deviation. For comparisons involving a single independent factor, one-way analysis of variance (ANOVA) was applied, followed by appropriate post hoc multiple comparison tests when significant differences were detected. To evaluate the combined effects of treatment group (sham, single, repeated administration) and biochemical parameters, two-way ANOVA was additionally performed, allowing assessment of main effects and interaction effects between factors. The relative contribution of each source of variation was quantified, and interaction terms were reported where applicable. A p value < 0.05 was considered statistically significant.

4. Results

4.1. In vivo observations

Following DMAC administration, animals were monitored for general appearance, behavior, and signs of neurological impairment throughout the experimental period. No mortality was observed in any experimental group. No overt neurological deficits, such as seizures, abnormal gait, paralysis, or loss of righting reflex, were detected in any group.

Rats receiving repeated administrations of 1000 mg/kg DMAC exhibited transient reductions in spontaneous activity following dosing but remained responsive to external stimuli. Animals receiving a single intraperitoneal dose of 2000 mg/kg DMAC showed marked clinical distress, including lethargy, reduced mobility, and decreased food intake. Due to these findings, repeated dosing at the 2000 mg/kg level was not pursued for animal welfare reasons. No significant change in body weight relative to baseline was observed in any group during the study period.

4.2. Biochemical findings

White blood cell (WBC) counts exhibited variable responses to repeated DMAC injections. In the group that received a single-administration, WBC levels tended to increase, whereas a declining trend was observed in the groups that received two- and three-administrations. Lymphocyte, monocyte, and granulocyte counts increased in the single administration group. In contrast, lymphocyte counts in the two- and three-administration groups initially increased but declined in the final measurements. Compared to sham controls, exposure to DMAC caused fluctuations in lymphocyte levels. Platelet counts were reduced across all DMAC-treated groups relative to sham controls, accompanied by decreases in platelet distribution width (PDW), suggesting a potential deleterious effect of DMAC on platelet function and distribution (see Table 1).

Significant elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed in all DMAC-treated groups compared to sham controls (AST: sham vs single administration, $p = 0.0144$; sham vs double administration, $p = 0.0021$; sham vs triple administration, $p < 0.0001$). The single high-dose group (2000 mg/kg) also showed increased AST and ALT levels relative to sham controls, consistent with DMAC-induced hepatotoxicity, although repeated dosing was not performed in this group. The highest AST levels were detected in the triple-administration group ($p < 0.0001$). ALT levels followed a similar trend, although the magnitude of the change was less pronounced (sham vs. double administration, $p = 0.0025$; sham vs. triple administration $p = 0.0099$). Coagulation parameters—including aPTT, PT, and INR—did not differ significantly between groups, suggesting that repetitive DMAC administration does not affect these measures (see Fig. 1C). In addition, body weight remained stable relative to baseline in all groups throughout the study period, and no treatment-related weight loss was observed.

4.3. Radiological findings

No ischemic changes, cortical or parenchymal lesions, or other pathological signal alterations were observed between the groups. Overall, the MRIs of all rats exposed to DMAC were considered normal. Therefore, despite DMAC exposure under experimental conditions, imaging findings remained within normal limits (Supplementary Figure).

4.4. Histopathological findings

Histologic evaluation allowed an objective comparison of morphological changes in both hepatic and neural tissues. Histological analysis of the liver and kidney tissues from the control group revealed no pathological alterations. The hepatic architecture and renal structures (glomeruli and tubules) appeared normal, with well-preserved histological integrity (see Fig. 2A). In the group that received a single 2000 mg/kg injection, liver sections displayed preserved architecture, with only mild cytoplasmic vacuolisation. However, pronounced glomerular and tubular necrosis was evident in the kidneys of this group. Additionally, tubular dilatation, vascular congestion, and intratubular cast formation were observed (see Fig. 2B). Liver sections from the single 1000 mg/kg injection group showed localised areas of hepatocellular

Table 1
Hemogram parameters after 1000 mg/kg i.p. injection of DMAC through repetitive administration respectively. Sham indicates 1X PBS injection. Each hemogram result was measured before euthanasia under general anesthesia. DMAC: dimethylacetamide.

	Repeat of administration											
	Single				Twice				Triple			
	Sham	1	2	3	Sham	1	2	3	Sham	1	2	3
WBC	5.57	6.23	6.39	9.16	9.19	6.44	8.48	3.87	8.46	9.33	6.79	5.68
LYM	3.73	3.84	4.71	4.38	6.64	3.87	4.8	2.32	2.73	6.08	4.77	3.04
MONO	0.12	0.52	0.31	0.4	0.06	0.24	0.77	0.36	0.73	0.25	0.23	0.75
GRA	1.72	1.87	1.37	4.38	2.49	2.33	2.91	1.19	4.99	3	1.78	1.9
LY%	67	61.7	73.7	47.9	72.3	60.1	56.6	60	32.3	65.2	70.3	53.4
MONO%	2.2	8.3	4.9	4.3	0.6	3.7	9.1	9.3	8.6	2.7	3.4	13.2
GR%	30.8	30	21.4	47.8	27.1	36.2	34.3	30.7	59.1	32.2	26.2	33.4
RBC	7.69	7.93	7.93	9.23	7.78	7.91	8.53	8.27	7.31	9.05	8.58	7.79
HGB	13.2	13.8	13.3	15.5	13.8	13.8	14.4	14.2	12.9	15.6	14.4	12.7
HCT	36.45	40.26	38.84	42.37	39.5	40.64	40.2	39.14	37.24	43.78	40.86	36.95
MCV	47	51	49	46	50	51	47	47	51	48	48	47
MCH	17.1	17.4	16.8	16.8	17.5	17.4	16.9	17.2	17.6	17.2	16.8	16.4
MCHC	36.1	34.2	34.2	36.5	34.9	33.8	35.9	36.3	34.6	35.6	35.3	34.5
RDWc	17.1	17.9	18	17.4	17.7	18.3	18.4	17.1	17.2	17.3	17.8	18.1
PLT	878	616	560	433	671	373	616	690	726	668	377	514
PCT	0.58	0.42	0.38	0.29	0.47	0.23	0.39	0.47	0.47	0.43	0.25	0.33
MPV	6.6	6.8	6.7	6.7	7	6.1	6.4	6.7	6.5	6.4	6.7	6.5
PDWc	32.7	35.6	35	33.4	34.6	33	33.4	33.4	34.2	34.2	33.4	34.2

necrosis along with sinusoidal irregularity and dilatation. Kidney tissues from this group exhibited marked glomerular sclerosis, tubular necrosis, congestion, and intratubular casts (see Fig. 2C). The group receiving two 1000 mg/kg injections displayed more severe hepatic injury, characterised by widespread hepatocyte necrosis and vascular congestion. In the kidneys, extensive glomerular and tubular necrosis, sclerosis, congestion, and features of thyroidization were evident (see Fig. 2D). The group that received three injections of 1000 mg/kg, exhibited the most severe histological damage. Liver sections showed severe necrosis and loss of classical lobular organisation due to extensive degenerative changes. Kidney sections revealed diffuse glomerular sclerosis, tubular necrosis, congestion, and prominent tubular dilatation. The epithelium of the dilated tubules appeared markedly thinned (see Fig. 2E).

Further histological evaluation of the cerebrum and cerebellum in the control group revealed no pathological alterations. The cortical layers of the cerebrum appeared intact without signs of degeneration. In cerebellum sections, no cellular degeneration was observed in the molecular, ganglionic, or granular layers of the cortex (see Fig. 3A). Cerebrum sections from the single 2000 mg/kg injection group showed cellular degeneration predominantly in the upper cortical layers, accompanied by subarachnoid edema, capillary dilatation, and vascular congestion. In some areas, oedema and haemorrhage were pronounced. In the cerebellum, subarachnoid oedema, capillary dilatation, and congestion were evident (see Fig. 3B). Cerebrum sections from the single 1000 mg/kg injection group exhibited marked cellular degeneration, subarachnoid oedema, capillary dilatation, congestion, and multifocal haemorrhage. Cerebellum sections from the same group revealed severe subarachnoid oedema, capillary dilatation, congestion, and haemorrhagic areas (see Fig. 3C). Cerebrum sections from the group receiving two 1000 mg/kg injections revealed cellular degeneration, subarachnoid oedema, inflammatory cell infiltration, capillary dilatation, congestion, and haemorrhage. In the cerebellum, edema was prominent, especially in the stratum gangliosum layer (see Fig. 3D). Cerebrum sections from the three-dose 1000 mg/kg group showed the most extensive damage, including severe cellular degeneration, subarachnoid edema, capillary dilatation, congestion, and haemorrhage. Cerebellar sections also displayed significant oedema in the ganglionic layer, accompanied by Purkinje cell hyperplasia. Furthermore, severe subarachnoid oedema, capillary dilatation, congestion, and haemorrhage were consistently observed (see Fig. 3E).

5. Discussion

The current study was designed in response to a rare clinical case suggesting a link between exposure to DMAC and potential neurotoxicity. The study aimed to systematically evaluate the systemic and neurohistopathological effects of DMAC exposure using a controlled animal model. While the hepatotoxic effects of DMAC are well documented, its impact on the central nervous system has not been sufficiently explored in the literature. Previous experimental and clinical studies have consistently demonstrated marked hepatotoxicity, including dose-dependent hepatic necrosis, elevated transaminases, and metabolic disturbances associated with DMAC exposure (Hundley et al., 1994; Kinney et al., 1993; Malley et al., 1995; Silvia et al., 1994). Therefore, this study supplements the limited number of clinical observations reported thus far with experimental data.

The primary motivation for the study was the radiological involvement in the case, which was consistent with isolated cortical laminar necrosis. Cortical laminar necrosis may be caused by cerebral hypoperfusion (e.g., generalised hypotension due to cardiac arrest or severe anaemia), hypoxia, hypoglycaemia, or cyanide poisoning. However, the literature on these cases does not describe the findings as being isolated to the cortex. It is debatable whether the brain MR findings in this case were due to hypotension and hypoxia caused by the long coma in the tank, or to the toxic effect of DMAC. In hypoxic ischemic encephalopathy, involvement of the deep grey matter and perirolandic cortex alongside the cortex, is typically expected. However, the absence of involvement outside the cortex in this case suggests that it may be due to the direct effect of DMAC. This novel finding suggests that the direct effects of DMAC on the central nervous system should be further investigated.

In one of the few studies addressing the neurological effects of DMAC, Weiss et al. reported that increasing doses of the drug led to lethargy, coma, and distinctive neurophysiological disturbances. These

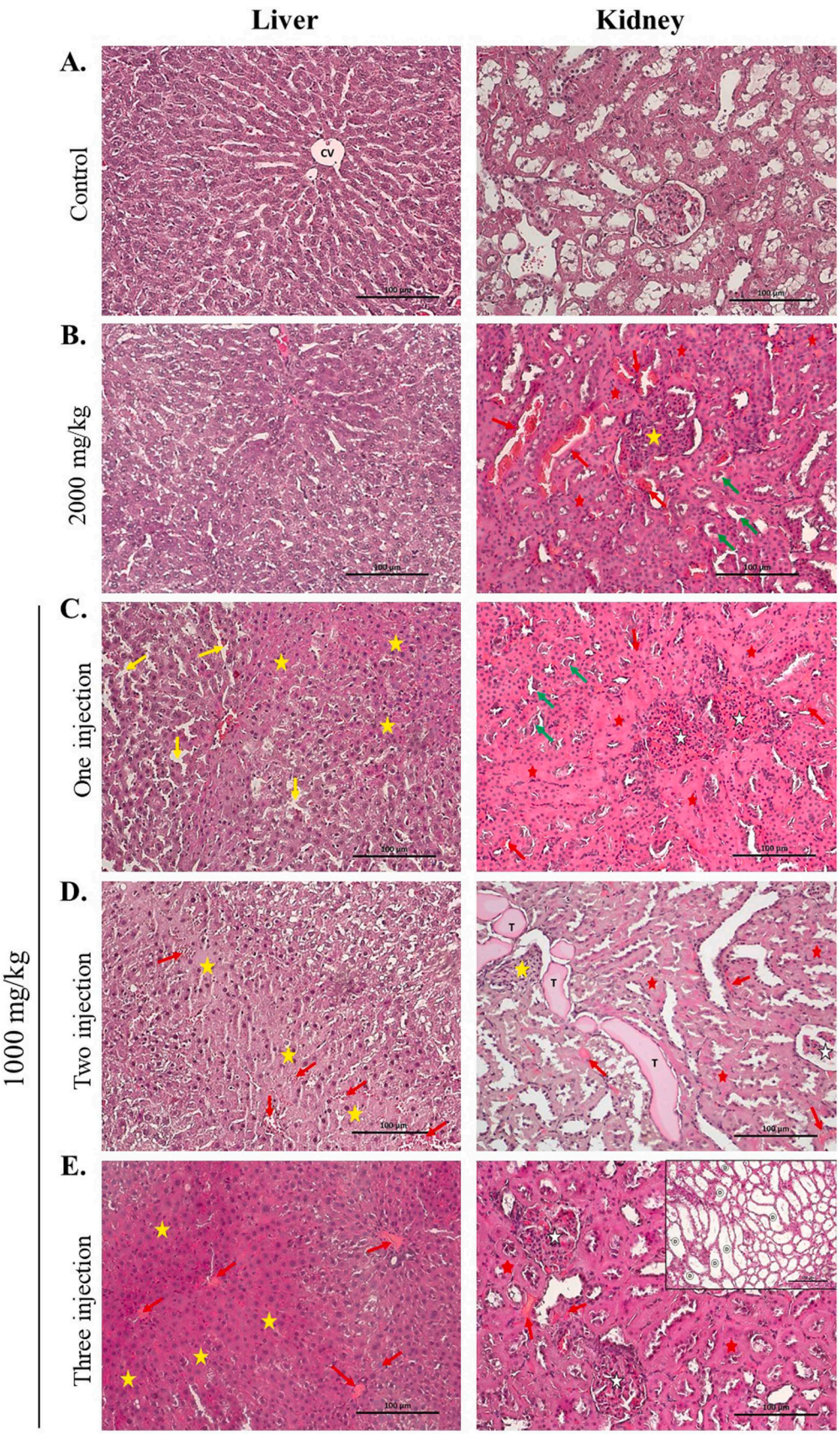

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Fig. 2. A. Liver and kidney sections from the control group. Liver and kidney histology appeared normal. CV: Central vein. **B.** Liver sections from the 2000 mg/kg single injection group showed normal histology, except for mild vacuolization. Kidney sections revealed glomerular (yellow asterisks) and tubular (red asterisks) necrosis. Additionally, vascular congestion (red arrows) and intratubular cast formation (green arrows) were observed. **C.** Liver sections from the 1000 mg/kg single injection group showed necrotic cell clusters in some areas (yellow asterisks), along with sinusoidal irregularity and dilatation (yellow arrows). Kidney sections from the same group exhibited severe glomerular sclerosis (white asterisks), tubular necrosis (red asterisks), congestion (red arrows), and intratubular cast formation (green arrows). **D.** Liver sections from the two-dose 1000 mg/kg group showed hepatocyte necrosis (yellow asterisks) and vascular congestion (red arrows). Kidney sections from the same group exhibited severe glomerular sclerosis (white asterisks), glomerular and tubular necrosis (yellow and red asterisks, respectively), congestion (red arrows), and thyroidization (T). **E.** Liver sections from the three-dose 1000 mg/kg group showed extensive necrosis (yellow asterisks) and congestion (red arrows). Classical hepatic lobulation was no longer distinguishable due to degenerative changes. Kidney sections from the same group showed glomerular sclerosis (white asterisks), tubular necrosis (red asterisk), and congestion (red arrows). The upper right inset demonstrates tubular dilatation (D) with markedly thinned epithelial lining. (H&EX200).

included episodic, moderately high-voltage slow waves and well-formed visual–sensory hallucinations on electroencephalograms (EEGs), primarily involving the frontoparietal regions (Weiss et al., 1962). Although these findings derive from a chemotherapy context, they provide important early clues suggesting that DMAC and structurally similar compounds may exert direct neurotoxic effects. In contrast to these early neurophysiological observations, large-scale occupational exposure data have not consistently demonstrated neurological involvement. In a recent outbreak investigation reported by Jiang et al., exposure levels to DMAC were well characterized in an industrial setting and were associated predominantly with hepatic and dermal toxicity; however, no neurological symptoms or neuroimaging findings were reported (Jiang et al., 2024). This distinction is notable, as it suggests that while systemic toxicity is the most commonly observed consequence of occupational DMAC exposure, neurological involvement may occur under specific exposure conditions. The isolated cortical involvement observed in the present case, together with characteristic magnetic resonance imaging findings, supports the possibility of a direct neurotoxic effect of DMAC on the cerebral cortex.

Beyond controlled therapeutic administration, occupational data have demonstrated that DMAC can be absorbed efficiently through both inhalational and dermal routes, creating significant potential for systemic toxicity when protective measures are insufficient. Indeed, several documented cases of occupational poisoning have shown severe clinical manifestations even at relatively low exposure levels (Jiang et al., 2024). More recently, analyses of industrial exposure incidents have confirmed that workers may experience substantial hepatic and metabolic injury despite airborne concentrations remaining within regulatory limits, highlighting that internal dose does not always correlate with ambient measurements (Jung et al., 2007; Klimisch and Hellwig, 2000). These findings underscore the relevance of dermal uptake and cumulative internal burden in real-world environments.

In addition to clinical reports, biomonitoring studies conducted in industrial settings have further clarified the relationship between external exposure and internal dose. A study of spandex factory workers demonstrated a strong correlation between airborne DMAC levels and urinary concentrations of its primary metabolite, N-methylacetamide, suggesting a creatinine-corrected threshold of approximately 20 mg/g for biological monitoring (Jung et al., 2007). Similarly, research from an acrylic fiber production facility showed that DMAC metabolites accumulated in workers' urine even when measured air concentrations were low, emphasizing that dermal absorption plays a major role in total body burden. Importantly, simple hygiene practices—such as showering after shifts and changing work clothes—significantly reduced these metabolite levels, illustrating that relatively minor interventions can meaningfully decrease systemic exposure (Perbellini et al., 2003). Taken together, these earlier studies provide a critical context for interpreting the neurological and systemic findings of the current experimental model, reinforcing that DMAC exposure—whether therapeutic or occupational—can reach biologically significant levels capable of producing both neurotoxic and systemic toxic effects.

Although no significant radiological results were obtained in the rats in our experimental model, the histopathological findings were apparent and revealing. Cerebral histopathological sections, in particular, showed degeneration of cells predominantly in the upper cortical layers, accompanied by subarachnoid edema, capillary dilatation, and vascular congestion. These findings clearly demonstrate that repeated administration of DMAC, even at a lower dose of 1000 mg/kg, results in cumulative neurohistopathological damage, primarily affecting cortical and subcortical structures in both the cerebrum and cerebellum. The most severe alterations to the brain were observed in the group that received three repeated doses of the substance.

The systemic toxicity induced by DMAC administration in rats was comprehensively evaluated in vivo, based on hematological, biochemical, and histopathological parameters, depending on the dose and frequency of administration. The findings suggest that repeated applications of DMAC cause significant cumulative tissue damage, primarily in the liver, kidneys, and central nervous system, which is consistent with systemic biological responses. Histological examinations revealed limited pathology in liver and kidney tissues following a single dose of DMAC. However, repeated doses resulted in distinct lesions emerging, including hepatocyte necrosis, lobular irregularity, glomerular sclerosis, tubular necrosis and thyroidization. In cerebral and cerebellar tissues, findings such as cortical layer degeneration, subarachnoid edema, capillary dilation, and hemorrhage increased with dose. The most severe neurohistopathological findings were observed in the group that received three doses. Overall, these results suggest that repeated administration of 1000 mg/kg of DMAC leads to cumulative histopathological damage in liver and kidney tissues.

In contrast, a single injection of 2000 mg/kg resulted in limited hepatic alterations, yet still caused notable renal injury. This suggests that the frequency of exposure plays a critical role in tissue toxicity. This comparison shows that the frequency of exposure is as important as the dose in determining tissue toxicity. These results show that DMAC has the potential to cause toxicity in both peripheral and central tissues, and that the frequency of exposure is a critical factor in determining these effects. It should be noted that occupational exposure limits expressed in ppm reflect airborne concentrations and cannot be directly converted to mg/kg doses used in experimental models. Intraperitoneal administration was therefore employed to ensure controlled and reproducible systemic exposure, allowing for the investigation of dose-dependent neurotoxic effects independent of variability in inhalation or dermal absorption.

The hemogram results demonstrate the efficacy of DMAC application on the hematopoietic and immune systems. Conversely, WBC and lymphocyte levels exhibited an upward trend after a single dose, but a downward trend was observed in subjects who received two or three doses. This finding indicates that the initial inflammatory response may have evolved into a state of immunosuppression following repeated exposure. The substantial decrease in PCT suggests that DMAC may have detrimental effects on megakaryocyte function or platelet lifespan. The observed decrease in platelet counts coincides with histologically evident vascular congestion and areas of bleeding.

Furthermore, the biochemical findings are highly indicative of liver damage. Concurrent elevations in AST and ALT levels correspond with histopathological observations of hepatocyte necrosis and sinusoidal irregularities. The group that received three doses exhibited the most widespread hepatic damage and had the highest levels of AST. Although

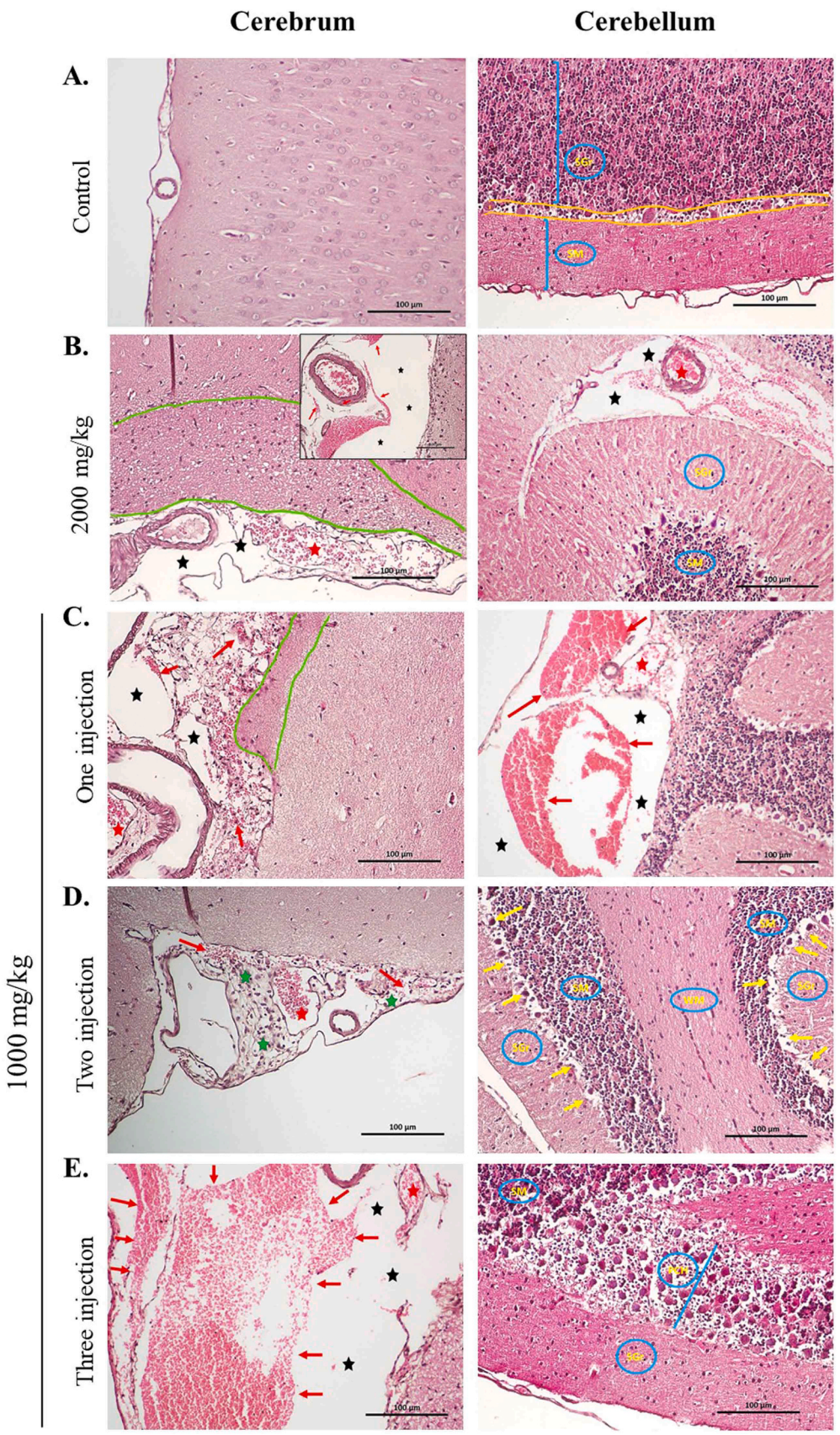

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Fig. 3. A. Cerebrum sections from the control group appeared normal. Cerebellum sections were also normal. No cellular degeneration was observed in the molecular (SM), ganglionar (area bounded by yellow line), or granular (SGr) layers of the cortex. **B.** In the 2000 mg/kg single injection group, cerebrum sections showed cellular degeneration, particularly in the upper cortical layers (area bounded by green line), along with subarachnoid edema (black asterisks), capillary dilatation, and congestion (red asterisk). The upper right inset highlights the area where edema (black asterisks) and hemorrhage (red arrows) are most prominent. In cerebellum sections, subarachnoid edema (black asterisk) and congestion (red asterisk) were also noted. SM: molecular; SGr: granular layers of the cortex. **C.** In the 1000 mg/kg single injection group, cerebrum sections exhibited cellular degeneration (area bounded by green line), subarachnoid edema (black asterisk), congestion (red asterisk), and hemorrhage (red arrows). Cerebellum sections from the same group showed severe subarachnoid edema (black asterisk). **D.** In the group receiving two 1000 mg/kg injections, cerebrum sections revealed cellular degeneration (area bounded by green line), inflammatory cell infiltration (green asterisks), congestion (red asterisk), and hemorrhage (red arrows). Cerebellum sections from this group showed edema in the stratum gangliosum (yellow arrows). SM: molecular; SGr: granular layers of the cortex; WM: white matter. **E.** In the group receiving three 1000 mg/kg injections, cerebrum sections displayed severe subarachnoid edema (black asterisk), congestion (red asterisk), and hemorrhage (red arrows). Cerebellum sections from this group demonstrated edema (yellow arrows) in the ganglionar layer and Purkinje cell hyperplasia (PCH). SM: molecular; SGr: granular layers of the cortex. (H&E, X200).

ALT levels also increased, they did not exhibit the same level of dramatic increase as AST, suggesting that DMAC may increase hepatocyte membrane permeability and cause damage, particularly of mitochondrial origin. In our patient, a moderate increase in AST levels was observed during follow-up, consistent with liver damage. The absence of significant differences between groups in coagulation parameters (PT, aPTT, and INR) suggests that DMAC does not affect the liver's ability to synthesize clotting factors indicating that hepatic dysfunction is limited to a subclinical level.

Overall, the in vivo stage reveals the potential for systemic toxicity of DMAC, showing that repeated doses can cause permanent tissue damage. One of the study's strengths in terms of its comprehensive toxicological evaluation is the consistency between the histological findings and the haematology and biochemistry results. Notably, the immune response changes that accompany liver and kidney damage suggest that DMAC exhibit both cytotoxic and immunomodulatory effects.

This study has some limitations. First, the study was designed as an exploratory in vivo toxicology investigation aimed at hazard identification rather than hypothesis-driven testing of predefined individual endpoints. Accordingly, the statistical *power analysis* performed during the ethical approval process was based on conservative assumptions suitable for exploratory designs and was not tailored to specific dependent measures. In addition, experimental dosing regimens, particularly at the higher DMAC concentration, were modified during the study in response to animal welfare considerations, which may have limited statistical power for specific comparisons. These factors should be taken into account when interpreting the findings. Additionally, occupational exposure to DMAC occurs mainly through inhalation and dermal contact, an inhalation-based experimental model was not employed. Such models require specialized exposure chambers and advanced biosafety infrastructure to ensure environmental and operator safety. Therefore, intraperitoneal administration was chosen to achieve controlled and reproducible systemic exposure. While this approach does not fully replicate real-world inhalational exposure, it allows the investigation of dose- and frequency-dependent neurotoxic effects under standardized conditions. In addition, circulating DMAC levels were not measured due to limited sample volume and the prioritization of comprehensive biochemical, hematological, and coagulation analyses. Taken together, the present findings indicate that DMAC exposure induces a multi-organ toxicity profile rather than isolated hepatotoxicity. In addition to well-established hepatic injury, significant renal histopathological alterations and distinct neurotoxic changes were observed in the experimental model. These results suggest that renal and central nervous system involvement represent integral components of DMAC-induced systemic toxicity and should be considered alongside hepatotoxic effects when evaluating its toxicological impact.

According to data reported by the National Institute for Occupational Safety and Health (NIOSH), the intraperitoneal median lethal dose (LD_{50}) of DMAC in rats is approximately 2750 mg/kg, and 2800 mg/kg in mice (U.S. Department of Health and Human Services, 1994). Furthermore, reproductive toxicity has been observed at doses as low as 2 mg/kg in pregnant rats, resulting in post-implantation mortality and fetotoxicity. These reference doses are often used to estimate risk thresholds in regulatory toxicology. However, discrepancies between such established LD_{50} values and the sublethal yet histopathologically evident toxic effects observed in experimental settings warrant further investigation. In our current study, significant hepatic, renal, and neurohistopathological alterations were observed at much lower repeated intraperitoneal doses of 1000 mg/kg, which are well below the reported LD_{50} thresholds. These findings suggest that while DMAC may not exhibit acute lethality at lower doses, cumulative or organ-specific toxicity may develop even within traditionally considered "safe" exposure ranges.

In conclusion, our clinical case and experimental data demonstrate that DMAC has significant neurotoxic potential. The observed neurotoxic effects should be interpreted within the context of a broader systemic toxicity profile that includes both hepatic and renal injury. Overall, our findings emphasize the potential of DMAC to cause cumulative, dose-dependent neurotoxicity, highlighting the need for stricter occupational safety measures and further experimental research to elucidate its mechanisms of brain involvement.

CRediT authorship contribution statement

Berna Yeniceri: Visualization, Methodology, Investigation. **Simay Bozkurt:** Visualization, Methodology, Investigation. **Sibel Atacan:** Visualization, Resources, Data curation. **Onder Huseyinbas:** Visualization, Methodology, Investigation. **Hizir Asliyuksek:** Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **Rumeysa Hekimoglu:** Writing – original draft, Visualization, Validation, Methodology, Investigation. **Nihan Hande Akcakaya:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Beyza Goncu:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Emrah Yucesan:** Writing – review & editing, Investigation, Conceptualization. **Mukaddes Esrefoglu:** Writing – review & editing, Visualization, Resources, Investigation, Formal analysis.

Ethical Statement

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Beyza Goncu reports financial support was provided by Bezmialem Vakif University. If there are other authors, they declare that they have no

known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2026.103392.

Data availability

Data will be made available on request.

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