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Int Cardiovasc Res J. 2026 December; 20(1): e166605 https://doi.org/10.69107/icrj-166605

Published Online: 2026 November 1 **Research Article**

Morphology, Viability, and Expression of Cardiac Markers in Human Cardiomyocytes After Exposure to Methamphetamine

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Received: 24 September, 2025; **Revised:** 19 September, 2026; **Accepted:** 17 October, 2026

Abstract

Background: Methamphetamine (METH), a highly addictive amphetamine derivative, is associated with severe cardiovascular complications, including cardiomyopathy. However, its precise effects on human cardiomyocytes (HCM) remain poorly understood.

Objectives: This study aimed to evaluate the biological activity of HCM, including viability and cardiac-related marker expression, after exposure to METH.

Methods: Cultured HCM were treated with 0.6 mM METH or phosphate-buffered saline vehicle. Cell morphology was assessed by inverted microscopy on days 1, 3, and 6. Viability was measured using the trypan blue exclusion method over 8 days. Expression of GATA-binding protein 4 (GATA4) and creatine kinase transcripts was measured by real-time polymerase chain reaction (PCR) on days 1, 3, and 6, with β-actin as the endogenous control. Experiments were performed in 3 biological replicates, each assessed in technical triplicate. Independent-samples t tests, analysis of variance (ANOVA), and post hoc tests were used for statistical comparisons between the METH-treated and vehicle-treated groups. A $P < 0.05$ was considered statistically significant.

Results: Unlike vehicle-treated cells, METH-treated HCM appeared unhealthy, with long, thin cytoplasmic processes on days 3 and 6. The number of viable cells increased transiently by day 4 but declined sharply by day 6 compared with vehicle-treated cells. GATA4 expression peaked significantly on day 1 (4.53 ± 0.74; 95% CI, 2.69 - 6.37; P = 0.011), decreased by day 3 (1.17 ± 1.43; 95% CI, 0.00 - 4.72), and was downregulated by day 6 (0.10 ± 0.23; 95% CI, 0.00 - 0.67) compared with the vehicle-treated group. *Creatine kinase* transcript expression showed a similar, statistically nonsignificant trend. Expression increased to 1.81 ± 0.71 (95% CI, 0.05 - 3.57) on day 1 and then was 1.47 ± 1.46 (95% CI, 0.00 - 5.10) on day 3. By day 6, expression had dropped sharply to 0.11 ± 0.38 (95% CI, 0.00 - 1.05).

Conclusions: Methamphetamine induces morphological changes, increases cell number, and dynamically alters the expression of cardiac-related markers in HCM, possibly reflecting early stress followed by dysfunction. These findings highlight changes in cardiomyocyte biological activity after METH exposure that may adversely affect cellular function. This study also demonstrates the potential of HCM models for investigating cardiomyopathy associated with exogenous substances.

Keywords: Methamphetamine, Cardiomyocytes, Morphology, Survival, Expression of Cardiac Markers

1. Background

Methamphetamine (METH), also known as “speed” or “crystal,” is a highly addictive amphetamine derivative that is widely abused for its ability to induce intense euphoria and increase wakefulness, as well as for other effects, such as decreased appetite (1-3). Its additional methyl group facilitates passage across the blood-brain barrier, contributing to its effects (4-6). Methamphetamine also impairs normal cardiovascular function. Associated complications include malignant hypertension, arrhythmias, heart failure, and myocardial infarction secondary to acute vasospasm unresponsive to vasodilator therapy. These cardiovascular complications constitute the second leading cause of mortality in METH overdose cases (7-9). Chronic METH exposure has also been reported to exacerbate myocardial damage (4, 7). Histological examinations and autopsy reports of long-term METH users have revealed hypertrophy, fibrosis, necrosis, and, ultimately, cardiomyopathy (10).

Nevertheless, the precise effects of METH on cardiac cells remain incompletely understood. Most studies of its

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How to Cite: Kargar Jahromi H, Roozbehi K, Ebrahimi-Zadeh F, Mirzaei V, Parsaei F, et al. Morphology, Viability, and Expression of Cardiac Markers in Human Cardiomyocytes After Exposure to Methamphetamine. Int Cardiovasc Res J. 2026;20(1):e166605. doi: https://doi.org/10.69107/icrj-166605

cardiovascular effects have used animal models or human autopsy findings (11, 12). Understanding the behavior of METH-treated human cardiomyocytes (HCM) may help guide appropriate interventions to counteract adverse effects. Cell models provide an opportunity to investigate the biological activity of HCM.

2. Objectives

This study aimed to evaluate morphology, viability, and the expression of cardiac-related markers, including GATA-binding protein 4 (GATA4) and the creatine kinase transcript, in HCM after exposure to METH.

3. Methods

This controlled in vitro experimental study used cultured HCM. Cells were obtained under the designation "HCM" (human cardiac myocytes) from a local branch of the Pasteur Institute (https://www.pasteur.ac.ir/; invoice number 10967; catalogue number 1578; NCBI code C599). These fibroblast-like cells were originally derived from heart tissue. Donor information was not provided by the Pasteur Institute. According to the supplier's quality-control documentation, the cells had been tested and confirmed to be free of mycoplasma, bacterial, and fungal contamination. Therefore, no in-house contamination testing was performed. The supplier did not specify whether the cells had been assessed for fibroblast contamination.

Human cardiomyocytes were cultured in a specialized medium containing Ham's F12 and DMEM (1:1, v/v), supplemented with insulin (5 µg/mL) and basic fibroblast growth factor (bFGF; 50 ng/mL), in a humidified atmosphere at 37 °C with 5% CO_2. Cells were passaged at 80% confluence using 0.05% trypsin/EDTA; a seeding density of 7500 cells/cm^2 was recommended for the subsequent passage. Passage-3 cells were used for the experiments. All experiments were performed in 3 biological replicates, each assessed in technical triplicate.

Human cardiomyocytes were cultured in T25 flasks until they reached 50% confluence. The METH-treated group was exposed to METH (Sigma-Aldrich, USA) at a final concentration of 0.6 mM in a total volume of 5 mL. Vehicle-control cells received culture medium with a matched phosphate-buffered saline (PBS) vehicle in a total volume of 5 mL. Cell morphology was evaluated using an inverted microscope equipped with a digital camera on days 1, 3, and 6. Viability was assessed using the trypan blue exclusion method. Briefly, METH-treated and vehicle-treated HCM were seeded in 24-well plates at 50 000 cells per well. After 24 h, cells from 3 wells in each plate were treated with 0.05% trypsin/EDTA. Following centrifugation at 1200 rpm for 10 min, a defined volume of the cell pellet was mixed with an equal volume of 0.04% trypan blue dye. Live cells were counted using a Neubauer counting chamber. This procedure was repeated for 8 consecutive days.

Cell groups were prepared in T25 flasks for gene expression analysis on days 1, 3, and 6. On each specified day, cells were harvested, and RNA was extracted from the cell pellets using RNX Plus solution according to the manufacturer's instructions (CINAGEN, Iran). RNA quality was assessed using the 260/280 and 260/230 absorbance ratios measured with a NanoDrop instrument. Genomic DNA contamination was removed by DNase I treatment following RNA extraction. RNA was then used as the template for cDNA synthesis (AddScript cDNA Synthesis Kit, Adbio, South Korea). Real-time polymerase chain reaction (PCR) was performed using RealQ_Plus 2x Master Mix Green (Ampliqon, Denmark) and specific primers for GATA4 and the creatine kinase transcript (Table 1). β-actin was used as the endogenous control. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method. Quantitative PCR efficiency was approximately 99%. No-template controls and no-reverse-transcription controls were included in every run.

Continuous variables were expressed as mean ± standard deviation (SD). The experimental unit for all quantitative analyses was an independent biological replicate. Technical triplicates were averaged to obtain a single representative value for each biological replicate before inferential statistical analysis. Because formal tests of normality and homoscedasticity have limited power at small sample sizes, data normality was visually assessed using distribution plots, and equal variance was assumed in accordance with standard cell culture analytical conventions. Statistical comparisons were performed using the independent-samples t-test or one-way analysis of variance (ANOVA), with Tukey's post hoc test for pairwise comparisons. All statistical analyses were conducted using SPSS Statistics, version 16 (IBM Corp., USA). A two-tailed $P < 0.05$ was considered statistically significant.

4. Results

4.1. Cell Morphology

Although the morphology of METH-treated and vehicle-treated cells did not appear to differ significantly over 6 days, long, thin processes extending from the cytoplasm were prominent in the METH-treated group. This appearance was particularly evident on day 3 and was absent in the vehicle-treated group. On day 6, METH-treated cells also appeared unhealthy compared with the

Table 1. Primer Sequences Used in Real-Time PCR [a]

Target	Forward (5'→3')	Reverse (5'→3')	Size (bp)
GATA4	CCCAATCTCGTAGATATG	GCTGAGGCTTGATGAG	172
Creatine kinase	ATGACCACTTCCTGTTC	ATCTCCTCAATCTTCTGC	223
β-actin	CTGGAACGGTGAAGGTGACA	GTCCTCGGCCACATTGTGAA	70

[a] Abbreviations: GATA4, GATA-binding protein 4; PCR, polymerase chain reaction; bp, base pairs.

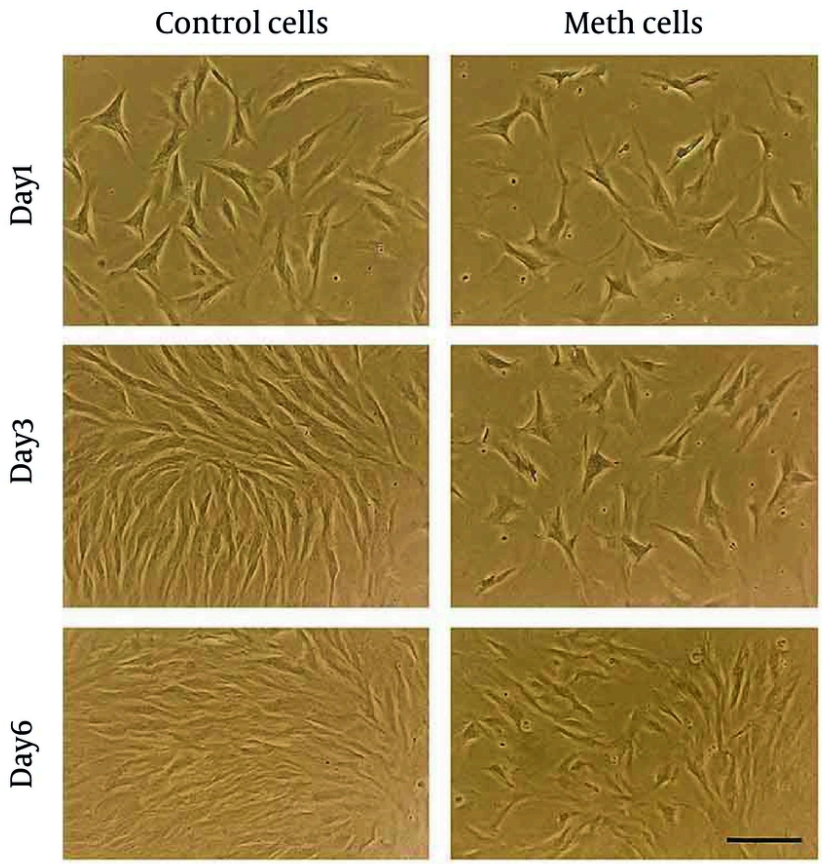


Figure 1. Morphology of methamphetamine-treated and vehicle-treated human cardiomyocytes over 6 days. Magnification: 360×; scale bar = 100 µm.

distinct fibroblast-like morphology characteristic of vehicle-treated HCM (Figure 1).

4.2. Cell Growth Curve

Growth curve analysis revealed that METH-treated HCM had a significantly increased viable cell number compared with vehicle-treated cells. This increase was more pronounced on day 4. However, the difference in cell number was statistically nonsignificant on all days. Vehicle-treated cells maintained an increasing growth pattern until day 6, whereas METH-treated cells experienced a sharp decline thereafter (Figure 2).

Table 2. Cardiac-Related Marker Expression Ratios in the METH-Treated Group Relative to Vehicle-Treated Controls [a]

Marker	Day 1	Day 3	Day 6	P value [b]	P value (days 1, 3) [c]	P value (days 1, 6) [c]	P value (days 3, 6) [c]
GATA4	4.53 ± 0.74	1.17 ± 1.43	0.10 ± 0.23	0.002	0.011	0.002	0.400
Creatine kinase	1.81 ± 0.71	1.47 ± 1.46	0.11 ± 0.38	0.152	-	-	-

[a] Values are expressed as mean ± SD. Control values were set to 1. Treatment-group values represent fold changes relative to controls, calculated using $2^{-\Delta\Delta Ct}$ analysis. Abbreviations: METH, methamphetamine; GATA4, GATA-binding protein 4; SD, standard deviation.

[b] P value indicates the comparison across days 1, 3, and 6.

[c] P value indicates comparisons between the specified pairs of time points. A $P < 0.05$ was considered statistically significant. This experiment was performed in 3 biological replicates, each assessed in technical triplicate.

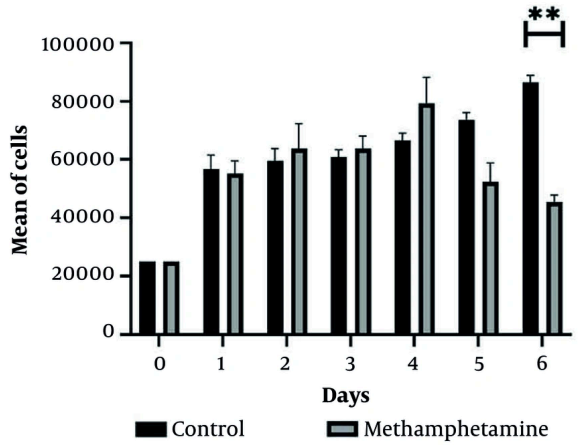


Figure 2. Growth pattern of cardiomyocytes treated with methamphetamine or phosphate-buffered saline vehicle. The experiment was performed in 3 biological replicates, each assessed in technical triplicate. A statistically significant difference between treated and vehicle-treated groups was evident only on day 6. Error bars represent the standard deviation. Analysis of variance (ANOVA) was used, with $P < 0.05$ indicating statistical significance.

4.3. Gene Expression

GATA4 expression peaked significantly on day 1 (4.53 ± 0.74; P = 0.011) in METH-treated HCM, decreased to near vehicle-treated levels on day 3 (1.17 ± 1.43), and was significantly downregulated by day 6 (0.10 ± 0.23; P = 0.002) compared with vehicle-treated cells. A significant difference in GATA4 expression was also observed between days 1 and 3 (P = 0.011). A similar descending pattern was observed for creatine kinase transcript expression, which increased to 1.81 ± 0.71-fold on day 1, followed by 1.47 ± 1.46-fold on day 3. By day 6, expression had dropped sharply to 0.11 ± 0.38. Differences in creatine kinase transcript expression between METH-treated and vehicle-treated cells were nonsignificant throughout the 6 days (P = 0.152) (Table 2). Treatment-group values are expressed as fold changes relative to controls and were calculated using the $2^{-\Delta\Delta Ct}$ method. Control values were normalized to 1. Comparisons were performed across days 1, 3, and 6 and between the specified pairs of time points; the corresponding P values are presented.

5. Discussion

This study examined the effects of METH on the biological activity of human cardiac cells. Morphology, growth kinetics, and expression of cardiac-related markers, including GATA4 and the creatine kinase transcript, showed notable changes in HCM following METH exposure. By day 3, METH-treated HCM exhibited long, thin cytoplasmic processes and, by day 6, an unhealthy appearance, unlike the typical fibroblast-like morphology of vehicle-treated cells. These changes are consistent with previous studies linking METH to oxidative stress, mitochondrial dysfunction, and disruption of autophagy-lysosome pathways in cardiac tissue (7, 13).

The growth curve showed a statistically nonsignificant increase in proliferative capacity by day 4, followed by a sharp decline by day 6, in contrast to sustained growth in vehicle-treated cells. This biphasic pattern is consistent with previous reports of early compensatory responses to METH-induced stress (2, 14), followed by cell death driven by senescence and apoptosis (7, 15). These findings support the hypothesis that cellular responses to METH toxicity follow a complex, multistage trajectory. Because cell number was determined using trypan blue exclusion and serial cell counting, the observed changes represent alterations in viable cell number and growth kinetics rather than direct evidence of increased cellular proliferation. The early increase in cell number may reflect changes in proliferation, survival, attachment, or other cellular processes.

The influence of METH on HCM was also investigated by examining cardiac-related gene expression. The

myocardial band isoenzyme of creatine kinase is found primarily in cardiac muscle and is released into the bloodstream following myocardial injury, making it a biomarker of cardiac damage (16, 17). GATA4 is another protein expressed by cardiomyocytes (18). A previous study showed that METH exposure causes cardiomyocyte senescence by increasing GATA4 levels (19). In the present study, GATA4 expression initially increased and subsequently decreased. *Creatine kinase* transcript expression also showed a trend toward change. Together, these findings suggest dynamic responses to METH, reflecting early stress and subsequent cellular responses in HCM. However, a retrospective study by Richards et al. suggested that the diagnostic utility of biomarkers in methamphetamine-associated cardiomyopathy (MACM) is likely comparable to that in the general heart failure population. Among METH-positive patients with elevated B-type natriuretic peptide levels, creatinine levels were significantly increased, whereas troponin I levels were not (20).

The METH concentration used in this study (0.6 mM) should be interpreted as an experimental exposure concentration rather than as a direct representation of circulating METH levels in humans. Previous in vitro studies investigating the direct effects of METH on cardiomyocytes have used concentrations ranging from micromolar to millimolar levels, including 0.05 - 1.0 mM in cultured adult rat ventricular cardiomyocytes (21) and 0.1 - 5.0 mM in isolated murine cardiomyocytes (2). These studies demonstrate that relatively high experimental concentrations can be used to investigate direct cellular responses to METH. However, concentrations measured in the blood of METH users are generally in the low-micromolar range (22). Therefore, the 0.6 mM concentration used here is substantially higher than typical circulating concentrations and should not be considered physiologically representative. Future dose-response studies incorporating a broader range of clinically and toxicologically relevant concentrations are required to determine whether these responses also occur at lower METH concentrations.

Previous studies have shown that the mechanisms underlying MACM are complex (23). Although they were not directly examined in this study, possible mechanisms include myofibrillar disorganization caused by sarcomeric protein degradation, mitochondrial dysfunction, impaired calcium homeostasis, disruption of cellular degradation systems, including the ubiquitin-proteasome system and autophagy-lysosome pathway, and altered myosin gene expression. These changes may promote cellular senescence and apoptosis (2, 13-15, 22). Such mechanisms impair contractile function and promote inflammation. Over time, persistent inflammation, myocyte loss, and replacement fibrosis lead to chamber dilation and reduced systolic function, manifesting clinically as dilated cardiomyopathy and heart failure with reduced ejection fraction (24-26).

Methamphetamine-associated cardiomyopathy is increasingly recognized as a distinct clinical entity amid the escalating METH epidemic (27, 28). Affected individuals are typically younger than those with cardiomyopathy of other etiologies (28). These patients often present with advanced heart failure, characterized by reduced ejection fraction and New York Heart Association class III - IV symptoms (25, 29). Several case reports have documented acute myocardial infarction in patients with MACM, frequently attributed to vasospasm or coronary thrombosis in the absence of preexisting coronary artery disease (30, 31). Their severe presentation is often associated with a poor prognosis. Nevertheless, a key characteristic of MACM is the potential reversibility of cardiac dysfunction following sustained abstinence from METH use (32). Cessation of METH use for 1 year is associated with improved global longitudinal strain and a lower risk of heart failure-related hospitalization (33). Optimal management requires a multidisciplinary approach involving primary care providers, psychiatry, social services, and substance use counselors.

5.1. Limitations

This study has several limitations. First, although the in vitro model provides a controlled environment, it may not fully replicate the complex physiological conditions of chronic METH exposure in vivo. Second, only 2 cardiac-related markers were evaluated, which may not capture the full spectrum of molecular changes associated with METH-induced cardiomyopathy. Additional markers, including complementary cardiac and cell-death markers, should be measured. Third, the single METH concentration used may not reflect the range of doses encountered in human use, limiting insight into dose-dependent effects. Potential sources of bias should also be considered. Measurement and observer bias cannot be completely excluded because morphological assessment was performed without explicit blinding to treatment allocation. Experimental bias may arise from using a single source of HCM. Authentication of the cardiomyocyte preparation represents an additional limitation. The limited number of independent biological replicates may restrict statistical power and the assessment of run-to-run experimental variability.

5.2. Conclusions

This study demonstrates that METH exposure induces morphological changes, alters growth kinetics, and

dynamically modulates cardiac-related gene expression in HCM under in vitro conditions. Further studies are needed to clarify the underlying mechanisms and determine whether these findings translate into clinically relevant cardiovascular effects.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

Authors' Contribution: Study Concept and Design: H. K. G. and I. R. J.; Data Acquisition: K. R., F. E., F. P., V. K., and V. M.; Data Analysis and Interpretation: I. R. J.; Manuscript Drafting: K. R. and I. R. J.; Critical Revision for Important Intellectual Content: All authors; Statistical Analysis: I. R. J. and M. J. Z.; Administrative, Technical, and Material Support: H. K. J. and V. M.; Study Supervision: I. R. J.

Conflict of Interests Statement: The authors declare no conflicts of interest for this study.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This study was approved under the ethical approval code of IR.JUMS.REC.1402.033 by the Research Ethics Committees of Jahrom University of Medical Sciences.

Funding/Support: This study was supported in part by grant 5678 from Jahrom University of Medical Sciences.

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