INTRODUCTION

The integrity of the cardiac functions depends on the optimal heart-nervous system connections. The heart is innervated by the autonomic nervous system, classified into sympathetic and parasympathetic nervous systems. Despite the opposite functions of the two systems, to maintain homeostasis, sympathetic and parasympathetic systems play an optimal role in regulating cardiovascular functions (1). Parasympathetic cardiac innervation provides an inhibitory impact by slowing down the heart rate, the neural control of the arterial blood pressure is the outcome of the activities of both systems on the central and peripheral levels (2). In contrast, sympathetic innervation accelerates the ventricular contraction and increases the heart rate by influencing neurotransmitter norepinephrine.

Loss of catecholaminergic innervation of the cardiac tissues results in sympathetic neurodegeneration of the heart and is considered a cardiac dysautonomia mechanism (3). Interestingly, patients with Parkinson’s disease exhibit varying levels of cardiac denervation. However, in Parkinson’s disease patients with intimately related cardiac dysautonomia and neurodegeneration, various symptoms characterize each component. Cardiac dysautonomia in Parkinson’s disease refers to cardiac neurodegeneration, orthostatic hypotension, fatigue, decreased time to peak heart rate variability, shortness of breath at regular exercise, and reduced blood catecholamines, especially plasma norepinephrine (4, 5).

Several animal models of cardiac sympathetic dysautonomia and neurodegeneration are available to study and validate therapeutic targets for preclinical evaluation. MPTP (1-methyl-
4-phenyl-1,2,3,6-tetrahydropyridine) is a lipophilic neurotoxin widely used in Parkinson’s disease nigral dopaminergic loss in mice (6) and monkeys (7). It has the ability not only to cross the blood-brain barriers affecting brain stem nuclei the sympathetic autonomic nuclei such as rostroventrolateral medulla (RVLM) and nucleus tractus solitarii (NTS) in the lower hindbrain, causing desensitization of baroreceptor reflex and impaired heart rate variability (8) but also induces loss of cardiac sympathetic innervation (9). MPTP affects the heart and gut through catecholamine neuron degeneration and extends to chromaffin cells of the adrenal medulla (10). Loss of cardiac sympathetic nerve induced by MPTP has been previously demonstrated in rats by a daily dose (20 mg/kg/5 days, i.p.) to yield 100% loss of cardiac norepinephrine (11).

Citicoline or CDP-choline is a drug composed of a cytidine 5'-diphosphate moiety and choline, after being absorbed is hydrolyzed into cytidine 5'-diphosphate and choline, that can cross the blood-brain barrier. In the brain, these hydrolysis derived components recombined to form citicoline in the neurons increasing the brain content of phospholipids (12). Citicoline is a safe drug with negligible acute or chronic toxicity (13). Previous studies introduced the neuroprotective effect of cytidine-diphosphate-choline (CDP-choline or citicoline) against several neurological disorders like Alzheimer’s disease, ischemic stroke (14), spinal cord injury (15) and a recent study showed its protective effect in children after cardiac arrest (16). In the current study, we aimed to study the effects of citicoline on cardiovascular function in MPTP-treated albino rats as an animal model for cardiovascular dysautonomia.

MATERIALS AND METHODS

Animal maintenance

Twenty-four male albino rats, initially weighing 150 – 200 g, were received from the Ophthalmology Research Institute (Giza, Egypt). We kept the rats in conventional animal service-based housing. The rats were accommodated to normal standard conditions (temperature 24 ± 1°C, relative humidity 40 – 60%, and a 12:12 h light/dark photoperiods). Moreover, they were maintained in polycarbonated cages (3 rats/cage) to avoid isolation stress for one week before starting the experiment. The rats were kept under monitoring along the acclimatization period to exclude infectious rats. The animals were provided with a standard basal diet and water ad libitum.

The approval of animal procedures, Care, and Use of Laboratory Animals was guided based on the Ethics and Scientific Committee (Eight edition 2011), Department of Physiology, Kasr Al Ainy, Faculty of Medicine, Cairo University, Egypt.

Experimental design for in vivo work

Pathogen-free twenty-four male albino rats were divided into four groups (6 rats each). The first group was designated as a negative control (normal control) and received intraperitoneal (i.p.) saline injection for five consecutive days. The second group was a positive control group and received citicoline (Somazine drops October Pharma, 6th of October city - Egypt Under License of Ferrer International, SA, Spain) (250 mg/kg) by oral gavage for consecutive 20 days based on a previous study (17). The third and fourth groups treated with MPTP-HCL (Sigma, St Louis, MO, USA) (30 mg/kg, i.p.) for five consecutive days based on previous studies (18), followed by additional treatment with oral doses of citicoline (250 mg/kg) for only the fourth group, which continued for 20 days. Food, water, and behavioral changes were monitored periodically, and the body weights were recorded weekly. At the end of the study, all the rats were exposed to the cardiovascular system’s functional assessment before euthanasia.

In vivo functional assessment of the cardiovascular system

The functional measurements performed over two days separated by one day (to allow recovery). D1: Transthoracic echocardiography using ketamine and xylazine, D3: measuring blood pressure in conscious rats followed ECG under urethane anesthesia.

1) Transthoracic echocardiography

The assessment was performed by using echocardiography to evaluate the cardiac functions in vivo. The rats were anesthetized by injecting both ketamine-hydrochloride (25 mg/kg, bw, i.p.) and xylazine (5 mg/kg, bw, i.p.). Anesthesia was followed by removing the hairs from the chest’s anterior part and maintaining the rats on a specialized warming table to maintain normothermia.

The echocardiography system was equipped with a 10 MHz phased-array transducer (GE Healthcare’s Vivid, USA). 2D short-axis view of the left ventricle and M-mode tracings were recorded to measure ejection fraction (EF), fractional shortening (FS), left ventricular dimension in diastole (LVDd), left ventricular dimension in systole (LVSD), left ventricular posterior wall thickness during diastole (LVPWD), left ventricular posterior wall thickness during systole (LVPWS), stroke volume (SV) and heart rate (HR) were estimated. Cardiac output was calculated from SV and HR [CO = HR × SV]. Trans mitral Doppler flows (ratios of E and A velocities) were recorded in an apical 4-chamber orientation with the sample volume placed at the tips of the mitral leaflet.

2) Measurement of the arterial systolic blood pressure

Blood pressure of animals were measured by non-invasive blood pressure monitor (LE 5001 , LETICA scientific Instruments, Espania) from the tail of conscious rats by the tail-cuff technique.

The tail-cuff technique is a common, non-invasive and convenient way to measure systolic blood pressure in an animal model. The pulsation disappears as the tail-cuff is inflated and restart to pulsate when the tail-cuff is deflated, which equals systolic pressure. The cuff is connected to a tail-cuff sphygmonanometer, and the blood pressure is recorded on a chart (20). The rats were conscious during measurement, warmed at 28°C for 30 min in a thermostatically controlled heating cabinet (UgoBasille, Italy) to detect tail artery pulse accurately. The tail was passed from the heating cabinet through a miniaturized cuff, and a tail-cuff sensor was connected to an amplifier (ML125 NIBP, AD Instruments, Australia). The amplified pulse was recorded during automatic inflation and deflation of the cuff. Systolic blood pressure was defined as the cuff inflation pressure at which waveform become indistinguishable from baseline noise. The average of at least three measurements was taken at each occasion.

3) Electrocardiogram recordings

Following the systolic blood pressure assessment, the rats were anesthetized again with urethane (0.6 mL/100 gm, b.w., intramuscular), and then the electrocardiogram was recorded.

In vitro tissue processing and studies

Following the heart’s functional assessment, the rats were sacrificed under ether anesthesia; the thoracic aorta was removed for subsequent evaluation of vascular reactivity. The heart was
excised for biochemical measurements (cardiac tyrosine hydroxylase, norepinephrine, glucose transporter 1 (GLUT1), insulin receptor substrate 1 (IRS1), peroxisome proliferator-activated receptor γ co-activator-1 (PPARγ co-activator-1) (PGC-1), phosphatase and tensin homolog-induced kinase 1 (PHK1), carnitine palmitoyltransferase 1 (CPT1), uncoupling protein 2 (UCP2) and adenosine monophosphate-activated protein kinase alpha 2 (AMPKα2)).

Vascular reactivity studies of isolated aorta

The thoracic aorta was immediately excised and cut into sagittal rings 3 mm in length and transferred to organ chambers rinsed with 10 mL of freshly prepared Krebs-Henseleit solution (NaCl 118 mM, KCl 4.7 mM, CaCl$_2$ 2.5 mM, MgSO$_4$ 1.2 mM, NaHCO$_3$ 25 mM, KH$_2$PO$_4$ 1.2 mM, glucose 10 mM) maintained at 37°C, pH 7.4 and incubated within 95% O$_2$ and 5% CO$_2$. Between
every two hooks, the aorta’s rings were mounted, and the hooks attached to an isometric force transducer, which in turn connected to the system of data acquisition (Power lab 8SP, AD Instruments) for consequent recording the tension of 1 gm for 1 h (21). The phenylephrine response curve (10, 20 and 40 µg concentrations) on isolated rat ring was generated from all the groups. Moreover, the response curve of isolated rat’s aortic ring to cumulative doses of acetylcholine can be calculated by the percentage of relaxation induced by 20 µg acetylcholine divide by a submaximal dose of 20 µg phenylephrine-induced contractions for every group (Fig. 1).

**Quantitative-RT-PCR**

During sacrificing, the hearts were excised and flourished with phosphate buffered-saline and cut into several pieces; part of these cardiac pieces was rinsed in stabilizing reagent RNA later solution,  

Fig. 2. Modulatory effect of citicoline on transcriptional levels of MPTP-treated cardiac tissue rats. Cardiac tissues of MPTP-exposed rats showed significant (**P < 0.001) upregulation of UCP2 and AMPKa2, then significant (**P < 0.01) downregulation of GLUT1, IRS1, PGC1α, and PINK1 were also observed when compared to the normal control group. On the other hand, citicoline administration to MPTP-treated rats was significantly (**P < 0.01) modulated to be close to normal compared to MPTP control rats. In contrast, the mRNA of CPT1 expression was not different between the studied groups. The data were presented as mean ± SE.
Trichloroacetic acid. The homogenate was then centrifuged at 15000 rpm in a cooling centrifuge for 15 min. The supernatant was decanted in a chilled clean small tube for subsequent processing. Total RNA extraction was performed from homogenized 25 mg of stored tissues using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany). The RNA concentration was determined using a UV spectrophotometer (Optima SP-3000+, Jenway Inc., Stone, UK) (22).

**Table 1. Real-time qPCR primer sequences.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Product size</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPKa2</td>
<td>forward 3' GGCCCTCTAGTCTCCATCCATCAG ATGTCAACGGTTGCTCTG reverse 5'</td>
<td>219</td>
<td>NM_006252.4</td>
</tr>
<tr>
<td>IRS1</td>
<td>forward 3' TCCTATCCCGGAAGGGGTCT TGGGCATAAGGCCATCATCA reverse 5'</td>
<td>103</td>
<td>NM_005544.3</td>
</tr>
<tr>
<td>SLC2A1 (GLUT1)</td>
<td>forward 3' ATGTCTATCTGAGCATGTT GTTGCTCCACATGCTGGAAC reverse 5'</td>
<td>193</td>
<td>NM_006516.4</td>
</tr>
<tr>
<td>PGC-1a</td>
<td>forward 3' CTACCGTGTACACCTGAGCG TTGTTGATCTAGCTGCA reverse 5'</td>
<td>140</td>
<td>NM_001330753.2</td>
</tr>
<tr>
<td>PINK1</td>
<td>forward 3' TGCCCCAACAACGAGGAAC ACGTGCTGACCCATGTTGATG reverse 5'</td>
<td>95</td>
<td>NM_032409.3</td>
</tr>
<tr>
<td>UCP2</td>
<td>forward 3' ACAAGACCTTGCAAGGAG ATGAGTTGGCTTTAGGAGG reverse 5'</td>
<td>142</td>
<td>NM_001381944.1</td>
</tr>
<tr>
<td>CPT1b</td>
<td>forward 3' CACGGACAGGAGTAAGCT CTTGAGACGATAAGGGTGCCG reverse 5'</td>
<td>546</td>
<td>NM_004377.4</td>
</tr>
<tr>
<td>ACTB (β-actin)</td>
<td>forward 3' TCTGCCCTTGGCCGATCC TCTCCATGTCGGCCAGTGTTG reverse 5'</td>
<td>298</td>
<td>NM_001101.5</td>
</tr>
</tbody>
</table>


**Table 2. Bodyweight and biochemical parameters of cardiac tissue in the studied groups.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Citicoline</th>
<th>MPTP</th>
<th>MPTP + Citicoline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline body weight (g)</strong></td>
<td>165 ± 6.708</td>
<td>168.33 ± 6.571</td>
<td>165.2 ± 6.830</td>
<td>166.57 ± 8.176</td>
</tr>
<tr>
<td><strong>Body weight at the end of the study (g)</strong></td>
<td>184.33 ± 6.103</td>
<td>189 ± 6.89</td>
<td>150.5 ± 5.396*</td>
<td>163.17 ± 7.101</td>
</tr>
<tr>
<td><strong>Tyrosine hydroxylase activity (nm/mg/h)</strong></td>
<td>19.68 ± 0.567</td>
<td>19.83 ± 1.175</td>
<td>14.48 ± 0.854*</td>
<td>16.52 ± 1.016**</td>
</tr>
<tr>
<td><strong>Norepinephrine (µg/g tissue)</strong></td>
<td>1.0430 ± 0.01738</td>
<td>1.0635 ± 0.01152</td>
<td>0.660 ± 0.05657*</td>
<td>0.877 ± 0.04464**</td>
</tr>
</tbody>
</table>

**MPTP group** showed significant (P < 0.001) weight loss compared to the control group. Citicoline therapy to MPTP-treated rats attenuated weight loss; however, it was not significantly effective compared to the MPTP group. Mitigation effect of citicoline on MPTP-treated cardiac tissues biochemical parameters: the tyrosine hydroxylase activity and norepinephrine were significantly (P < 0.05) reduced in MPTP-treated rats' cardiac tissues compared to the control. On the other hand, citicoline administration to MPTP-treated rats showed a significant (P < 0.01) increase compared to MPTP-treated rats. The data were presented as mean ± SE. Body weight 1: at the start of the work; body weight 2: at the end of the work.

and other pieces were freshly cleaned and then stored at –80°C for subsequent processing. Total RNA extraction was performed from homogenized 25 mg of stored tissues using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany). The RNA concentration was determined using a UV spectrophotometer (Optima SP-3000+, Japan) at 260 and 280 nm. Reverse transcription was performed for 1.5 µg of RNA using MiScript II RTPCR kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. A quantitative RTPCR was performed with qPCR System (Life Technologies, Carlsbad, CA, USA) and QuantiTect SYBR Green Master Mix (Qiagen, Hilden, Germany). All the experiments were performed in triplicate, and the results were normalized to a housekeeping gene. The sequences of used primers are listed in Table 1.

**Fluorometric determination of norepinephrine in the myocardial tissue**

The freshly stored cardiac pieces were weighed and homogenized using a Teflon pestle homogenizer (Thomas Scientific, Swedesboro, NJ, USA) in 10 volumes of 5% of trichloroacetic acid. The homogenate was then centrifuged at 15000 rpm in a cooling centrifuge for 15 min. The supernatant was decanted in a chilled clean small tube for subsequent processing. Total RNA extraction was performed from homogenized 25 mg of stored tissues using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany). The RNA concentration was determined using a UV spectrophotometer (Optima SP-3000+, Japan) at 260 and 280 nm. Reverse transcription was performed for 1.5 µg of RNA using MiScript II RTPCR kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. A quantitative RTPCR was performed with qPCR System (Life Technologies, Carlsbad, CA, USA) and QuantiTect SYBR Green Master Mix (Qiagen, Hilden, Germany). All the experiments were performed in triplicate, and the results were normalized to a housekeeping gene. The sequences of used primers are listed in Table 1.

**Tyrosine hydroxylase (HT) activity**

The assay was performed by the simple fluorescence assay technique previously described (23). The dopa enzyme’s rapid reaction was carried out by a double column procedure (the top column of Amberlite CG-50 and Aluminum oxide on the bottom column). The Amberlite CG-50 column was used for erasing the interfering substances, while the second column of aluminum oxide for dopa adsorbance. The dopa was then eluted using 0.5 M acetic acid and determined by the previously described hydroxy indole method (24).

**Statistical analysis**

The experiments were performed in triplicates. The data were expressed by mean ± standard error (SE) and were statistically analyzed using the Student's t-test.
statistically analyzed using GraphPad v.6.0.0 software (GraphPad, San Diego, USA). We checked normality of distribution by Shapiro-Wilk test. The data were analyzed using two-way ANOVA and three-way ANOVA (post-hoc Tukey’s test) to compare the mean differences between the experimental groups.

**RESULTS**

**Body weight**

At the end of the work, MPTP group showed significant (P < 0.001) weight loss compared to control group. Citicoline

\[ \text{Fig. 3. Citicoline regulatory effect on echocardiography of MPTP-exposed rats. The presented data elucidated during the functional assessment of the heart. There is a significant (**P < 0.01) reduction of ejection fraction, functional shortening, and E/A ratio in MPTP-treated rats (A, B) when compared to normal control. Administration of citicoline regulates and elevates these events to be close to normal control compared to the MPTP-treated control group. The data were presented as mean ± SE.} \]
therapy to MPTP-treated rats attenuated weight loss however it was not significant effect compared to MPTP group (Table 2).

Modulatory effect of citicoline on biochemical parameters of cardiac tissues

The represented data in Table 2 show that tyrosine hydroxylase and norepinephrine were significantly decreased in the MPTP group compared with the normal control group. The administration of citicoline significantly (**P < 0.01) increased EF, FS, LVd, HR, SV, and CO compared to MPTP group. No significant difference was found in LVsd and LVPWS between the studied groups. The data were presented as mean ± SE.

**Abbreviations:** EF, ejection fraction; FS, fractional shortening; LVd, left ventricular internal diameter during diastole; LVsd, left ventricular posterior wall thickness during diastole; LVPWS, left ventricular posterior wall thickness during systole; IVSS, interventricular septal wall thickness during systole; IVSD, interventricular septal wall thickness during diastole; CO, cardiac output.

Regulatory effect of citicoline on the electrocardiography recordings

The presented data in Table 3 and Fig. 3 show the relevant echocardiography readings during the heart’s functional assessment. There was a significant (**P < 0.01) reduction of EF, FS, E/A ratio, LVd, LVPWS, HR, SV and CO in MPTP-treated rats when compared to normal control. Administration of citicoline significantly (**P < 0.01) increased EF, FS, LVd, HR, SV and CO compared to MPTP group. No significant difference was found in LVsd and LVPWS between the studied groups. The data were presented as mean ± SE.

Implication of citicoline on vascular reactivity-based aortic rings contractions

The vascular reactivity of the aortic rings’ contraction in response to various phenylephrine doses has been presented in
The results indicated a significant (P < 0.001) decrease in the contraction force in the MPTP-treated group at doses 10, 20, and 40 µg of phenylephrine when compared to the normal control group. On the other hand, there is a significant (P < 0.001) recovery of the contraction force of the aortic ring contraction at the indicated doses of phenylephrine in the MPTP-treated group with citicoline near to normal levels of the control group. There is a significant elevation in the aortic rings’ contraction force in a dose-dependent manner in all the studied groups.

**DISCUSSION**

The present study investigated the cardiovascular system’s chemical and mechanical dysfunctions during the experimental model of dysautonomia-induced rats using MPTP. Previous studies used an experimental model of Parkinson’s disease by using MPTP (25, 26). The main chemical dysfunction observed in the cardiovascular myocytes of MPTP-treated rats were depletion of tyrosine hydroxylase activity, norepinephrine secretion, decrease the relative mRNA transcription of GLUT1, IRS1, PPARγ co-activator-1, PGC-1, PINK1. Furthermore, and increased the relative mRNA transcription of uncoupling protein 2 (UCP2) and adenosine monophosphate-activated protein kinase alpha 2 (AMPKα2). Moreover, the significant mechanical dysfunctions recorded in cardiac myocytes of MPTP-treated rats were reduced fractional cell shortening, prolongation of P-wave duration relaxation, and decreased velocities of both shortening and re-lengthening. The administration of citicoline has modulated the above abnormalities.

The evidence of the anatomical studies suggested that the cardiovascular system’s sympathetic innervation is affected in neurodegenerative disorders, including synucleinopathies, which develop Lewy body’s formation, and Parkinson’s disease (27).
Fig. 5. Ameliorative impact of citicoline on arterial blood pressure of MPTP-treated rats. The MPTP-exposed rats showed a significant (** P < 0.01) reduction of systolic, diastolic blood pressure, mean arterial blood pressure, and heart rate compared to the normal control group. Contrary, citicoline administration to MPTP rats revealed a significant (** P < 0.01) recovery of mentioned events compared to the MPTP-treated rats. The data were presented as mean ± SE.

Fig. 6. Regulatory effect of citicoline on aortic ring contraction of MPTP-treated rats. The contraction force of aortic rings showed a significant (** P < 0.01) decrease in MPTP-exposed rats at the indicated doses of phenylephrine compared to the normal control group. On the contrary, the citicoline administration of MPTP-exposed rats showed a significant (** P < 0.01) recovery close to normal control rats. The data were presented as mean ± SE.
The current study documented that the cardiac system’s sympathetic denervation occurred in MPTP-treated rats (28) and was modulated upon administration of citicoline. Other previous studies confirmed our results that shown depletion of cardiac norepinephrine in MPTP-treated rats (29). Ren et al., studies (25) demonstrated that the number of β-adrenergic receptors is likely to share the decreased adrenergic responsiveness and eventually impair mechanical cardiac functions in MPTP-treated mice. Even though sympathetic denervation may lead to postsynapses’ supersensitivity due to lack of neural uptake of norepinephrine, various denervation-based-supersensitivity models remain controversial. Besides, Amino et al., (30) demonstrated that the cardiac nerve fibers and terminals were morphologically well preserved in the MPTP-treated mice, despite cardiac sympathetic dysfunction indicated by the significant decreases in the contents of cardiac noradrenaline and dopamine. Furthermore, MPTP fundamental impact focuses on its ability to cross the blood-brain barrier after application to MPP+, and this active form selectively enters the postganglionic sympathetic nerves through monoamine transporter (9). The MPP+ eventually recruited in mitochondria resulted in catecholamine neurodegeneration.

It is noteworthy that citicoline administration regulated cardiac catecholamine levels near the normal control, matched with previous investigations (31). The tyrosine hydroxylase activity in the dopaminergic neurons has been reduced in MPTP-treated rats and then increased near to normal level upon citicoline administration. The critical effect of citicoline could be due to the regulation of neuroinflammatory signaling.

Multiple studies reported that citicoline protects from heart damage caused by ischemia/reperfusion processes by reducing mitochondrial permeability. The results indicated the regulation of oxidative phosphorylation and cis-aconitate mitochondrial enzyme, which results in avoidance of oxidative disruption of mitochondrial DNA and the release of cytokines (32). The protective effect of citicoline upon the dopaminergic system has been investigated previously based on its pharmacological actions (33).

The most dominant heart problems found in the current research were decreased cardiac contraction and P waves’ prolongation during the time of relaxation, associated with the reduced maximal velocity of contraction/relaxation in MPTP-treated rats. Moreover, depletion of systolic, diastolic blood pressure, heart rate, and mean arterial blood pressure in MPTP-exposed rats were also observed. These results were confirmed by previous studies that reported that changes of heart rate variability through electrocardiographic assessment in Parkinson’s disease model was used MPTP (34, 35). Various factors may have disrupted the heart’s mechanical functions, including impaired functions of contractile proteins like actin and myosin isofoms, decreased availability of intracellular Ca²⁺ due to affected myofilament Ca²⁺ sensitivity, despite other alternatives that may exist but remain to be unclear.

The electrocardiogram can also be used in isolated Langendorff rat hearts, as demonstrated by Regev et al. (36) in their study that evaluated the ventricular arrhythmia and the interobserver agreement on ventricular fibrillation. Furthermore, Bernikova et al. (37) evaluated the electrical activity by EEG in reperfusion arrhythmogenesis and correlated it to the duration of repolarization using tetrodallylammonium (TEA) that blocks outward potassium currents and pinacidil that opens ATP-sensitive potassium channels.

The current study showed evidence of cardiac function dysregulation using the MPTP-animal model. Upregulation of mitochondrial protein; uncoupling protein 2 family observed in MPTP-treated rats due to excessive production of reactive oxygen species which activated UCP2 transcription to regulate and control the excessive ROS production through negative feedback (38). The function of AMPK in the heart is not cleared yet and become active during stress-induced hypertrophy. The present investigation showed upregulation of the AMPKα2 transcription in the MPTP-treated group. Furthermore, the increasing of AMPKα2 mediates the adiponectin’s anti hypertrophic effects (39). The AMPK can also influence the metabolic activity of gene expression. It plays a role in skeletal muscle exercise by increasing the peroxisome proliferator activated-receptor γ-coactivator-1α (PGC-1α) similar to what happened in our study; it was increased to upregulate the transcription of PGC-1α and Glut1 to regulate the mitochondrial oxidation pathways (40).

In the same perspective of observing metabolic and inflammatory markers in cardiovascular diseases Badacz et al. (41) showed in their study an association between proinflammatory cytokines, chemokines and the atherosclerotic plaque with internal carotid artery stenosis.

Citicoline is involved in patients’ protection of memory disorders against the excessive generation of oxidative stresses. In the present study, citicoline contributed to cardiac recovery when administered to MPTP-treated rats. The citicoline functions improved the mitochondrial dysfunctions and mitophagy by ameliorating UCP2, AMPK-α2 transactions (42). Several studies introduced valuable evidence on citicoline’s protective effect upon myocardial dysfunction during the rats’ heart reperfusion (25). Similarly, citicoline proved a protective function against calcium retention through modulation of PGC-1α, which regulates the oxidative metabolism and biogenesis of mitochondria for cardiac dysautonomia (43).

Additional studies demonstrated that citicoline aid in the synthesis of phosphatidylcholine that is a vital structure of cell membranes and mediators of cell signaling. Another likely mechanism of the protective effect of citicoline in the present study is its action to block inflammation by inhibiting phospholipase A2. This enzyme is involved in the breakdown of membrane phospholipids into arachidonic acid. The oxidative metabolism of arachidonic acid contributes to the generation of neuroinflammation and reactive oxygen species (ROS). By blocking phospholipase A2, citicoline may contribute to the reduction of inflammation, ROS formation, and neuronal damage (44). Citicoline causes an increase in SIRT1 (silent information regulator 1, SIRT1). SIRT1 belongs to the histone deacetylase family and regulates metabolic homeostasis and neuronal aging (45). In the cardiovascular system, activation of SIRT1 can only protect at the cellular level against oxidative stress, but also offer increased survival at the systemic level to limit coronary heart disease and cerebrovascular disease (46).

The present study evaluated the effect of citicoline therapy on cardiovascular function and investigated some biochemical markers’ levels. Citicoline improved cardiac contractility, electrical activity, blood pressure, and vascular reactivity. Citicoline also increased cardiac norepinephrine and tyrosine hydroxylase and improved markers related to ROS scavenger, mitochondrial permeability, calcium homeostasis on the cellular level, metabolic homeostasis, and mitochondrial biogenesis. Further studies are needed using electron microscopy and investigating other levels as post transcriptional or translational levels required to validate the changes of the metabolic markers (UCP2, AMPKα2, GLUT1, IRS1, PGC1α, PINK1, and CPT1) through immunohistochimetry or Western blot analysis for better evaluation of the citicoline’s cellular and molecular effects on cardiovascular autonomic dysfunction.

**Availability of data:** The data that support the findings of this study are available from the corresponding author (S.N. Amin).

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