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Original Research

# Protective Role of Sarpogrelate in Combination with Bromocriptine and Cabergoline for Treatment of Diabetes in Alloxan-induced Diabetic Rats

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## ARTICLE INFO

## Article history:

Received 28 July 2021

Accepted 30 September 2021

## Keywords:

Cardiovascular  
 Diabetic nephropathy  
 Dopamine receptors  
 Myocardial injury  
 Sarpogrelate

## ABSTRACT

**Background:** Although dopamine D<sub>2</sub> receptor agonists, bromocriptine and cabergoline, are notable medications in the treatment of Parkinsonism, hyperprolactinemia, and hyperglycemia, there is an identified relationship between the utilization of D<sub>2</sub>-like R agonists and the progress of myocardial injury, especially in the early phase of therapy.

**Objective:** This investigation aimed to examine the potential activity of sarpogrelate (a 5-hydroxytryptamine 2A [5-HT<sub>2A</sub>] receptor blocker) in reducing myocardial injury prompted by extended haul utilization of D<sub>2</sub> receptor agonists in a model of diabetic rats.

**Methods:** In the in vivo studies, both bromocriptine and cabergoline were managed independently and combined with sarpogrelate for about a month in diabetic nephropathy rats. Blood glucose level and other myocardial biochemical parameters were estimated. The probable mechanism for insulin secretagogue action was evaluated through in vitro isolated islets study. Sodium/potassium-adenosine triphosphatase activity was assayed in an isolated microsomal fraction of the renal cortex. Isolated perfused rat hearts were treated with different doses of dopamine before and after being subjected to the tested drugs, dose response of heart rate, and heart contractility were recorded.

**Results:** Bromocriptine and cabergoline created a significant reduction in blood glucose level without any action on insulin secretagogues. Bromocriptine prevented the loss of sodium/potassium-adenosine triphosphatase activity in the cortex of an ischemic kidney. Treatment of bromocriptine or cabergoline with sarpogrelate altogether decreased the levels of the elevated myocardial biomarkers in serum. Administration of different doses of dopamine in presence of bromocriptine or capergoline resulted in significantly rising in the heart rate percentage comparing to dopamine alone. A mix of bromocriptine or cabergoline with sarpogrelate diminished both heart rate and contractility, respectively.

**Conclusions:** The examination demonstrated that the combined use of sarpogrelate with bromocriptine or cabergoline decreased the potential adverse effects of these 2 drugs on myocardial tissues.

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## Introduction

Recently, dopamine D<sub>2</sub> receptor agonists are shown to be very important class of drugs for treatment of different diseases. Some of its important examples, bromocriptine and capergoline, are widely used for treatment of Parkinsonism and hyperprolactinemia. The antihypertension activity of dopamine D<sub>2</sub> receptor agonists are reported by deactivation of sympathetic tone, vasodi-

lation action, and alteration of sodium<sup>+</sup>/potassium<sup>+</sup>-adenosine triphosphatase (Na<sup>+</sup>/K<sup>+</sup>-ATPase) activity.<sup>1,2</sup> The role of bromocriptine for decreasing the ischemia-reperfusion injury of the kidney was identified and its mechanism believed to increase the phosphorylation of p44/42 mitogen-activated protein kinase in the proximal tubule. Studies show that activation of dopamine D<sub>2</sub> receptors causes stimulation of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in the proximal tubules of the kidney and prevent chronic nephropathy.<sup>3,4</sup> The nephron-protective effect of bromocriptine with better preservation of renal function as measured by creatinine clearance was observed in some previous studies,<sup>5,6</sup> and there are some experimental studies reporting the treatment with bromocriptine prevents or retards the development of lupus nephropathy in mice.<sup>7,8</sup>

Currently, bromocriptine expands the range of treatment options for type 2 diabetes. This might optimize the beneficial clinical effect of blood glucose control in those who respond poorly to standard agents. Recently, bromocriptine was approved for use in the market for the management of type 2 diabetes without significant risk of hypoglycemia.<sup>9</sup> Several mechanisms of bromocriptine's action have been proposed, but the precise mechanism of action remains unclear in most of the former reports. One previous report stated that bromocriptine shows ability for decreasing levels of dopamine centrally through its action on hypothalamus followed by decreasing the sympathetic tone, leading to decrease in the production of glucose from the liver and stimulate insulin release.<sup>10</sup>

Despite the medical benefits of dopamine D<sub>2</sub> agonists for treatment of various diseases, there are some reports that identified a relationship between the chronic use of some of these drugs, like bromocriptine, cabergoline, and pergolide, and development of significant myocardial injury and valvular stenosis.<sup>11-13</sup> In addition, it was established that long-term use of bromocriptine in a cumulative dose-dependent manner was associated with high risk of myocardial disease.<sup>14</sup> There is no direct relationship between activation of dopamine D<sub>2</sub> receptor and the initiation of myocardial injury because stimulation of D<sub>2</sub> receptors are reported to have numerous actions, represented in inhibiting adenylyl cyclase followed by the blockage of voltage-gated calcium ions and activating conductance of potassium showing no cause for arrhythmia or palpitation.<sup>15</sup> Most dopamine D<sub>2</sub> receptor agonists are classified as ergot and nonergot dopamine D<sub>2</sub> agonists. Ropinirole and pramipexole, examples of nonergot-derivative drugs, have few reported events of myocardial disease because they have no action on serotonin 5-hydroxytryptamine [5-HT] 2A or 5-HT<sub>2B</sub> receptors.<sup>16-18</sup> Bromocriptine and cabergoline, examples of ergot-derivative drugs, are linked to initiation of cardiomyopathy in patients because this type of drug has agonistic properties toward D<sub>2</sub> and 5-HT<sub>2A</sub> receptors. The reported cases of myocardial infarction were markedly increased in patients taking bromocriptine or cabergoline, but not for patients taking nonergot-derived drugs.<sup>19</sup>

Previous studies identified the role of the serotonergic system in the development of myocardial injury and the activation of the 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors as the main cause of the excessive cellular proliferation of cardiofibroblasts and the development of cardiomyopathy.<sup>20,21</sup> Currently, there are several dopamine D<sub>2</sub> agonist drugs, used for different medical proposals, that show high affinity toward 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors, including bromocriptine and cabergoline. Because of this, most of the studies concerned the safety of long-term treatment with cabergoline or bromocriptine in patients with hyperprolactinemia or Parkinson disease. There are not enough data to determine whether or not a low dose of bromocriptine or cabergoline, as are commonly used in patients with prolactinoma, is associated with clinically significant heart disease.

Sarpogrelate and ketanserin, selective 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> antagonists, are reported to reduce myocardial infarction area, cardiac trauma, and the electrocardiac changes resulting from my-

ocardial injury. Reports support the idea that serotonin and 5-HT<sub>2A</sub> or 5-HT<sub>2B</sub> receptors may play the main role in the development of ischemic myocardial injury.<sup>22,23</sup> The current study chose to study sarpogrelate, a selective 5-HT<sub>2A</sub> antagonist, because it has been approved for the treatment of peripheral vascular disease and shown to be relatively safe for use. Sarpogrelate hydrochloride, marketed as Anplag, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan, has been used to treat patients with peripheral arterial disease in Japan, China, and South Korea.<sup>24</sup> Sarpogrelate has shown significant effect in the treatment of myocardial infarction, peripheral vascular sickness, coronary artery spasm, and pneumonic hypertension.<sup>25</sup> The basis of the combinations of drugs in this study depends on using selective serotonin 5-HT<sub>2A</sub> antagonist drugs such as sarpogrelate to antagonize the identified serotonergic activity of bromocriptine and cabergoline on 5-HT<sub>2A</sub>, which represents the main reason for its adverse effects on myocardial tissue and the only pharmacological action of the tested drugs will be apparently related to its dopaminergic D<sub>2</sub> agonistic activity. The present study aimed to determine the possible therapeutic effect of sarpogrelate, a 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors blocker, associated with the cumulative use of dopamine D<sub>2</sub> receptor agonist drug therapy on myocardial and renal functions in a rat model of diabetic nephropathy.

## Materials and Methods

### Materials

Sarpogrelate and cabergoline were obtained from Sigma Aldrich Chemie GmbH, St. Louis, Missouri, USA. Bromocriptine was sourced from Novartis, Milano, Italy. Glibenclamide was obtained from Spimaco (Jeddah, Kingdom of Saudi Arabia). Alloxan, fetal bovine serum, and Dulbecco's modified Eagle medium were obtained from Sigma-Aldrich (St. Louis, Missouri). Trypsin and Hanks' balanced salt solution (HBSS) were collected from HyClone Laboratories (Thermo Fisher Scientific, Waltham, Massachusetts). Insulin ELISA kit (ab100578, lot: GR3279516-1), cardiac troponin 1 ELISA kit (ab246529, lot: GR3309533-1), rat CKM ELISA kit (ab187396, lot: GR327198-1), and ATPase assay colorimetric kit (ab234055, lot: GR3298881-1) were obtained from Abcam (Cambridge, UK). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Gold Biotechnology (St. Louis, Missouri).

### Animals

Forty-two Wistar albino male rats aged 5 to 7 weeks weighing 150 to 200 g were obtained from the animal house of Batterjee Medical College, Jeddah, Kingdom of Saudi Arabia. Three rats were housed per cage under controlled standard laboratory conditions in monitored ventilated cages and spontaneously given food and water. The ethical committee of research at Batterjee Medical College approved the steps of the investigation. Tutelage was taken, particularly with relevant habitation conditions, to minimize the animals' discomfort and the rats were kept on sawdust-floored cages. Data were coded before analysis so that the treatment group could not be identified before the analysis was completed. By the end of the study, all rats were killed by thiopental (150 mg/kg) for tissue collection.

### Experimental design

Diabetes was induced in the rats by a single dose of alloxan (150 mg/kg). The rats that showed a plasma glucose level of more than 250 mg/dL were considered to be diabetic and taken into the study.<sup>26</sup> Diabetic rats with a settled, recorded rise in the levels of urea and creatinine in the blood were collected. The sam-

ples were calculated based on the resource equation method.<sup>27</sup> The diabetic rats were divided randomly into 6 diabetic groups and 1 healthy control group, each group comprising 6 rats. Diabetic control group, and diabetic groups were treated with bromocriptine (4 mg/kg IP),<sup>28</sup> cabergoline (0.6 mg/kg IP),<sup>29</sup> and sarpogrelate (50 mg/kg IP),<sup>30</sup> respectively. Dopamine D<sub>2</sub> agonists' bromocriptine and cabergoline were used to induce myocardial infarction in the rats. The drugs were administered daily doses at 4 mg/kg and 0.6 mg/kg IP, respectively, in 2 groups of rats for 1 month. A dose of 50 mg/kg IP sarpogrelate with bromocriptine and cabergoline, respectively, was administered daily in 2 groups of rats for 1 month for testing its potential protective effect on the heart. Blood glucose levels were estimated on the seventh, 14th, 21st, and 28th days from the beginning of the drug treatment using an advanced glucometer (Roche, Indianapolis, Indiana).<sup>31</sup>

#### Multiple cardiac biomarker assays

At the end of the treatment, the isolated blood samples were centrifuged 3000 g for 15 minutes and the serum was frozen at -40°C until assayed for concentrations of creatine kinase MB (CKM) and cardiac troponin I (cTnI). The myocardial injury was defined by estimating CK-MB and cTnI levels in serum. Rat cardiac troponin I ELISA kit (Abcam), and rat CK-M-type ELISA kit (Abcam) were used for measurement of serum levels of cTnI and CKM.<sup>32,33</sup> Both serum CK-MB and Tn I levels were quantitatively measured by means of a highly specific enzyme immunoassay according to the manufacturer's instructions and procedures.

#### Isolation of rat pancreatic islets

The method of trypsin-EDTA digestion was used for isolation of pancreatic islets.<sup>34</sup> The pancreases were removed from normal overnight-fasted rats under aseptic conditions. The isolated pancreatic tissues were cut into small parts and washed with Hanks' balanced salt solution. The process of digestion was carried out by soaking the tissues into cold dissociation solution composed of 0.25% trypsin, and 0.1% EDTA with continuous shaking for about 20 minutes. After the removal of most of the enzymatic fluid, 2 mL warmed Dulbecco's modified Eagle medium at 37°C was added to the residual trypsin solution with continuous shaking for about 10 minutes. The process of digestion was ended by addition of 5% fetal bovine serum with gentle shaking and the suspension was pipetted and washed several times to maximize the process of cells isolation. Then, the suspension was centrifuged at 1000 rpm for 5 minutes at 4°C using refrigerated centrifuge model sigma 2-16pk (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The single-cell suspension was washed many times with HBSS. The isolated cells or islets were added to 10% fetal bovine serum in culture flasks and incubated at 37°C in a carbon dioxide incubator (Daihan LabTech Co, Batam, Indonesia) to be ready for the further study.

#### Viability assessment by MTT conversion

The viability of the normal isolated cells or islets lonely or after treatment with the tested drugs was assessed by the method of MTT assay. The solution of MTT was 0.5 mg/mL to be added into each well containing islets, and then incubated in a carbon dioxide incubator for about 5 hours. Then the islets were washed followed by the addition of 200 µL Dimethyl sulfoxide (DMSO) to dissolve any formed crystals and incubated for 1 hour. The absorbance was measured at 630 nm on a microplate reader (Biotek ELx800, Winooski, Vermont, USA). The viability of cells was expressed in percent viability relative to untreated cells considered 100% viable.<sup>35</sup>

#### Insulin release assay

Groups of 10 islets were placed in wells each containing 1 mL (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HBSS) (pH 7.4) supplemented with 10 mmol/L HEPES and 2 mg/mL Bovine Serum Albumin (BSA). Cells were then incubated for 1 hour with 11.8 mM glucose to be a negative control.<sup>36</sup> Glibenclamide (20.24 µM) was used as a positive control. Cells were incubated for 1 hour with 11.8 mM glucose in the presence of bromocriptine (3.66 mM), cabergoline (797 nM), sarpogrelate (69.84 mM), a mix of bromocriptine and sarpogrelate (1:1), and a mix of cabergoline and sarpogrelate (1:1). After incubation, the supernatant from each well was collected and stored at -20°C until further use. The insulin concentration in all of the stored samples was determined using an insulin ELISA kit (Abcam) and quantified using an ELISA microplate reader.

#### Renal tissue homogenates preparation

Immediately after blood collection, tested animals were humanely killed by lethal doses of thiopental sodium. The rats' abdomens were opened and right kidneys were quickly dissected and cut into small pieces. The kidney pieces were put in phosphate buffered saline, pH 7.4, containing 0.16 mg mL<sup>-1</sup> heparin to remove any red blood cells (erythrocytes) and clots. Some parts of the kidneys were homogenized with a stirrer homogenizer in cold phosphate buffer, pH 7.0 with EDTA.

#### Determination of Na<sup>+</sup>/K<sup>+</sup>-ATPase assay in renal tissue homogenate

A microsomal fraction was isolated from the renal cortex and medulla as previously described.<sup>37</sup> For tissue lysate, the isolated tissue (320 mg) was rapidly homogenized with 3.2 mL ice cold ATPase assay buffer using a small mixer homogenizer, and placed the sample on ice for few minutes. Centrifuge was undertaken at 8000 × g at 4°C for 10 minutes and the supernatant was collected. The endogenous phosphate in the collected tissue will overlap with Na<sup>+</sup>/K<sup>+</sup>-ATPase assay. Endogenous phosphate was removed by using ammonium sulphate method: aliquot the tissue samples (100 µL) to a clean Eppendorf tube, then ammonium sulphate was added to a final concentration of 3.2 M and placed on ice for few minutes. We spun down samples at 8000 × g at 4°C for 10 minutes, then the supernatant was discarded and the pellet was re-suspended back. Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was assayed by measuring the amount of inorganic phosphate liberated from ATP during incubation of the microsomal fraction in the presence of appropriate activators as reported in the ATPase Kit protocol (ATPase Assay Kit Colorimetric, code ab234055). The ATPase activity of the test sample was calculated by applying the optical density to the phosphate standard curve to get the amount of phosphate in the sample well (in micromoles) generated by ATPase during the reaction time (ie, 60 minutes). Enzyme-specific activity was expressed as micromoles phosphate released per milligram protein per hour.

#### Preparation of isolated hearts and testing the action of the drugs on the cardiac response

Normal Wistar male albino rats weighing between 150 and 200 g were injected with heparin sodium (8 mg/kg SC) 1 hour before they were humanely killed. The heart was rapidly removed and perfused by Langendorffs technique<sup>38</sup> with oxygenated buffer solution at a flow rate of 3 mL/min. The temperature of the perfusion fluid was 37°C and pH was 7.4. The composition of buffer solution was sodium chloride, dextrose, potassium chloride, calcium chloride (CaCl<sub>2</sub>·2H<sub>2</sub>O), magnesium chloride (MgCl<sub>2</sub>·6H<sub>2</sub>O), and sodium bicarbonate. The contractility was monitored by means

**Table 1**  
Effect of the tested drugs on blood glucose level (BGL) in rats with alloxan-induced diabetes.

BGL (mg/dL) <sup>†</sup>				
Group*	Week 1	Week 2	Week 3	Week 4
Normal control group	136.5 (18.52)	121.33 (12.08)	119.25 (11.35)	109 (10.92)
Diabetic control group	359.75 (35.13) <sup>‡</sup>	328 (14.34) <sup>‡</sup>	428.5 (12.17) <sup>‡</sup>	396.5 (10.60) <sup>‡</sup>
Diabetic group treated with bromocriptine	191 (16.89) <sup>§</sup>	199.25 (21.05) <sup>§</sup>	238.25 (26.88) <sup>§</sup>	284.25 (31.00) <sup>§</sup>
Diabetic group treated with cabergoline	213.75 (37.74) <sup>§</sup>	211.75 (22.62) <sup>§</sup>	293 (21.15) <sup>§</sup>	280.25 (22.48) <sup>§</sup>
Diabetic group treated with sarpogrelate	326.25 (13.45)	298.25 (23.37)	393.75 (18.77)	369.75 (20.15)
Diabetic group treated with bromocriptine + sarpogrelate	247.75 (29.35) <sup>§</sup>	215.5 (13.20) <sup>§</sup>	310.25 (17.59) <sup>§</sup>	288.25 (10.41) <sup>§</sup>
Diabetic group treated with cabergoline + sarpogrelate	196 (14.20) <sup>§</sup>	158.75 (15.01) <sup>§</sup>	235.5 (25.22) <sup>§</sup>	215.75 (18.46) <sup>§</sup>

\* n = 6 rats per group.

<sup>†</sup> Values are presented as mean (SEM).

<sup>‡</sup> P < 0.05, significantly different from the normal control group.

<sup>§</sup> P < 0.05, significantly different from the diabetic control group.

of a clip placed at the apex of the heart and connected to a digital heart lever. Both force of contraction and heart rate were recorded by LabScribe Software (Ugobasile Company, Gemonio, Lombardy, Italy). The heart was allowed to stabilize for 10 minutes before the addition of any drug. The responses to graded doses of dopamine 1, 5, 10, and 20 µg/mL were recorded.<sup>39</sup> In the second series of experiments the interaction of dopamine with bromocriptine, cabergoline, sarpogrelate, combined bromocriptine and sarpogrelate, and combined cabergoline and sarpogrelate was studied. Hearts were perfused for 10 minutes with the perfusion buffer solution containing bromocriptine (3.66 mM), cabergoline (797 nM), or sarpogrelate (69.84 mM). Thereafter, dopamine responses were recorded.

#### Statistical analysis

The statistical analysis of the data was done complied with the recommendations for experimental design and analysis in pharmacology.<sup>40</sup> The results were expressed as mean (SE). The significance of the differences between the values was performed by a 1-way ANOVA test and Tukey Kramer's Multiple Comparison Test using SPSS software (IBM-SPSS Inc, Armonk, New York). P values < 0.05 was considered significant.

## Results

#### Estimation of blood glucose levels and myocardial biomarkers

Administration of daily dose of both bromocriptine and cabergoline drugs, either alone or in mix with sarpogrelate, in the diabetic rats throughout 1 month of treatment showed a significant (P < 0.05) reduction in blood glucose levels relative to the untreated diabetic group. Administration of sarpogrelate individually for 1 month of treatment failed to show any action on the blood glucose levels (Table 1). There was a significant reduction in mean body weight of all diabetic groups (P < 0.05). However, bromocriptine and cabergoline treated groups showed a nonsignificant reduction in mean body weight compared with the diabetic control group. Rats treated with bromocriptine and cabergoline for 1 month in doses 10 mg/kg and 0.6 mg/kg, respectively, displayed significant elevation of CK-MB in serum. In contrast, it was observed that using a combination of bromocriptine and cabergoline with sarpogrelate significantly decreased the serum level of the CK-MB biomarker (Table 2). The results indicated that the quantitative test of the troponin I reagent kit showed only a significant elevation in cardiac troponin I in the bromocriptine- and cabergoline-treated groups. The groups administered a combination of bromocriptine and cabergoline with sarpogrelate showed

lower levels of myocardial biomarkers expression than the groups treated with bromocriptine or cabergoline individually (Table 2).

#### Insulin secretion studies on isolated pancreatic islets

The percentage of viability for the isolated islets exposed to 11.8 mM glucose alone were considered to have 100% viability and acted as the control group. The isolated islets treated with glibenclamide displayed viability of 94.33%. The viability of the isolated islets treated with the drugs under investigation was not highly affected. Bromocriptine, cabergoline, sarpogrelate, mix of bromocriptine and sarpogrelate, and mix of cabergoline and sarpogrelate showed 92.00%, 93.00%, 95.33%, 89.82%, and 90.12% viability, respectively.

The insulin secretagogue effect of each tested drug was assessed (Figures 1A and 1B). Among the 6 preparations of drugs (each at a concentration 10 µg/mL), glibenclamide showed a high insulin secretion (129.11 µIU) compared with the 11.8 mM glucose control. All of the tested drugs showed insulin secretion same as to 11.8 mM glucose control secretions.

#### Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in the cortical homogenates

The effect of the tested drugs on the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase in the kidney is presented in Table 3. Examination of the data reveal that the activity of the enzyme was significantly (P < 0.05) decreased in the kidneys of the diabetic group rats compared with control. Bromocriptine-treated group mice showed a significant increase enzyme activity. Both cabergoline and sarpogrelate failed to show any marked change in the activity of the enzyme. This result indicates that bromocriptine prevents the loss of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in the cortex of the ischemic kidney, as in the model of diabetic nephropathy in the current study.

#### Pharmacological action with dopamine and the tested drugs on the isolated hearts

The results are summarized in Table 4, and Figures 2A and 2B. Dopamine produced a dose-dependent increase in heart rate and positive inotropic action on isolated rat hearts. Both bromocriptine (3.66 mM) and cabergoline (797 nM) pretreatment markedly increased the chronotropic and the inotropic responses of heart to dopamine. Sarpogrelate (69.84 mM) pre-treatment failed to show any changes in the action of dopamine. Combination of sarpogrelate with bromocriptine or capergoline markedly decreased the positive chronotropic and inotropic responses of these drugs with dopamine (Figures 2A and 2B). Individually, both bromocriptine and cabergoline showed a significant increase in heart rate

**Table 2**  
Effect of the tested drugs on serum creatine kinase-MB-type (CK-M) and troponin I levels in alloxan-induced diabetic rats after 4 weeks of treatment.

Biochemicals <sup>†</sup>		
Group*	CK-M (pg/mL)	Troponin I (ng/mL)
Normal control group	36.23 (2.49)	0.41 (0.032)
Diabetic control group	41.50 (3.53)	0.49 (0.053)
Diabetic group treated with bromocriptine	75.50 (5.69) <sup>‡</sup>	0.73 (0.061) <sup>‡</sup>
Diabetic group treated with cabergoline	68.86 (4.47) <sup>‡</sup>	0.80 (0.065) <sup>‡</sup>
Diabetic group treated with sarpogrelate	38.15 (12.20)	0.38 (0.028)
Diabetic group treated with bromocriptine + sarpogrelate	47.75 (3.91) <sup>§</sup>	0.61 (0.054) <sup>§</sup>
Diabetic group treated with cabergoline + sarpogrelate	51.57 (4.90) <sup>  </sup>	0.67 (0.053) <sup>  </sup>

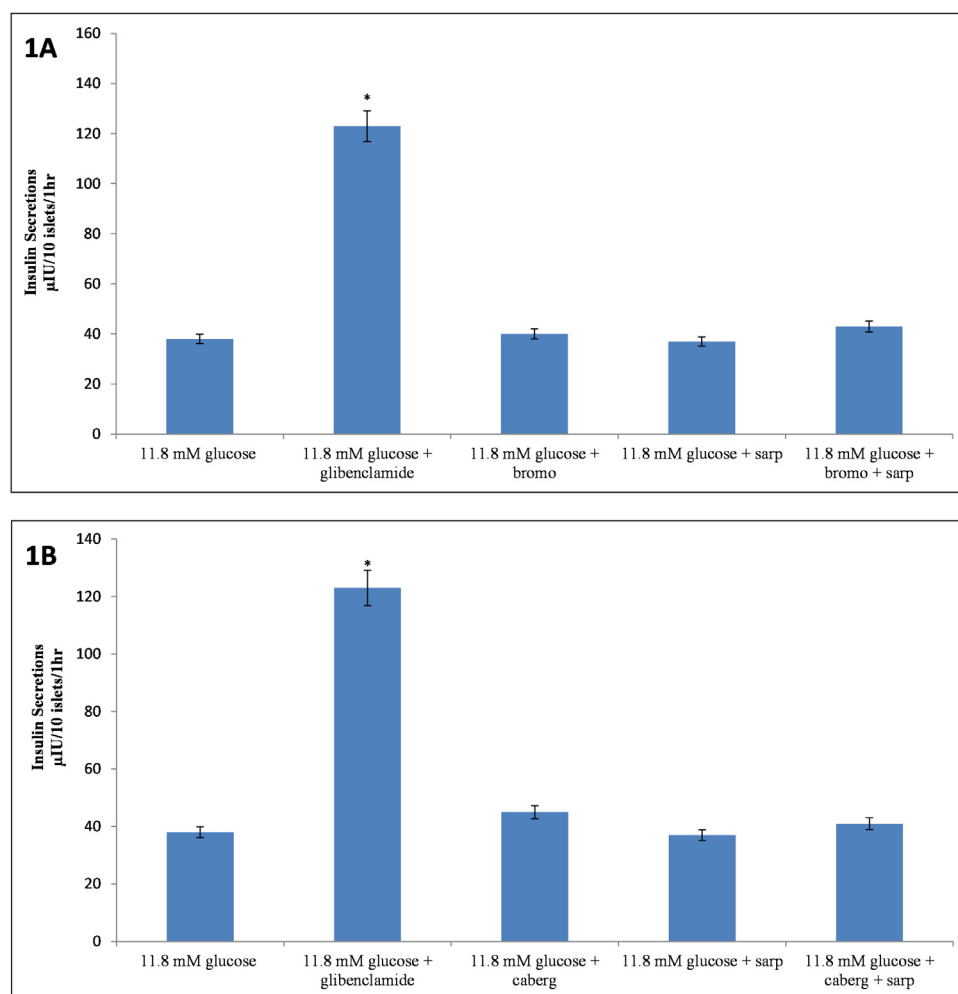
\* n = 6 rats per group.

<sup>†</sup> Values are presented as mean (SEM).

<sup>‡</sup> P < 0.05, significantly different from diabetic control group.

<sup>§</sup> P < 0.05, significantly different from diabetic group treated with bromocriptine.

<sup>||</sup> P < 0.05, significantly different from diabetic group treated with cabergoline.



**Figure 1.** Effect of the tested drugs on insulin secretion from isolated rat islets (n = 6). Insulin secretion induced by 11.8 mM glucose was considered as a negative control and glibenclamide as a positive control. Glibenclamide was used at a concentration 20.24 μM. Panel A shows the effect of bromocriptine alone at a concentration 3.66 mM and its combination with sarpogrelate (69.84 mM). Panel B shows the effect of cabergoline alone (797 nM) and its combination with sarpogrelate (69.84 mM) on insulin secretions. Results are presented as mean (SEM). \*P < 0.05 is significant from 11.8 mM glucose control.

and cardiac contractility, whereas sarpogrelate indicated a mild decrease in heart rate (Table 4 and Figure 3). Addition of sarpogrelate in mix with bromocriptine or cabergoline on isolated rat hearts significantly decreased heart rate and cardiac contractility as shown in Figure 3. Because both heart rate and contractility are a critical determinants of myocardial oxygen consumption, the relationship between heart rate and prognosis or severity of myocar-

dial ischemia was assumed. Elevated heart rate and increase cardiac contractility predicts risk for developing myocardial injury.

### Discussion

It has been reported that patients taking bromocriptine and cabergoline have an increased risk of developing valvular heart disease. Previous reports stated that patients with greater exposure

**Table 3**

Effect of the tested drugs on sodium-potassium ATPase pump activity in rats with alloxan-induced diabetes after 4 weeks of treatment.

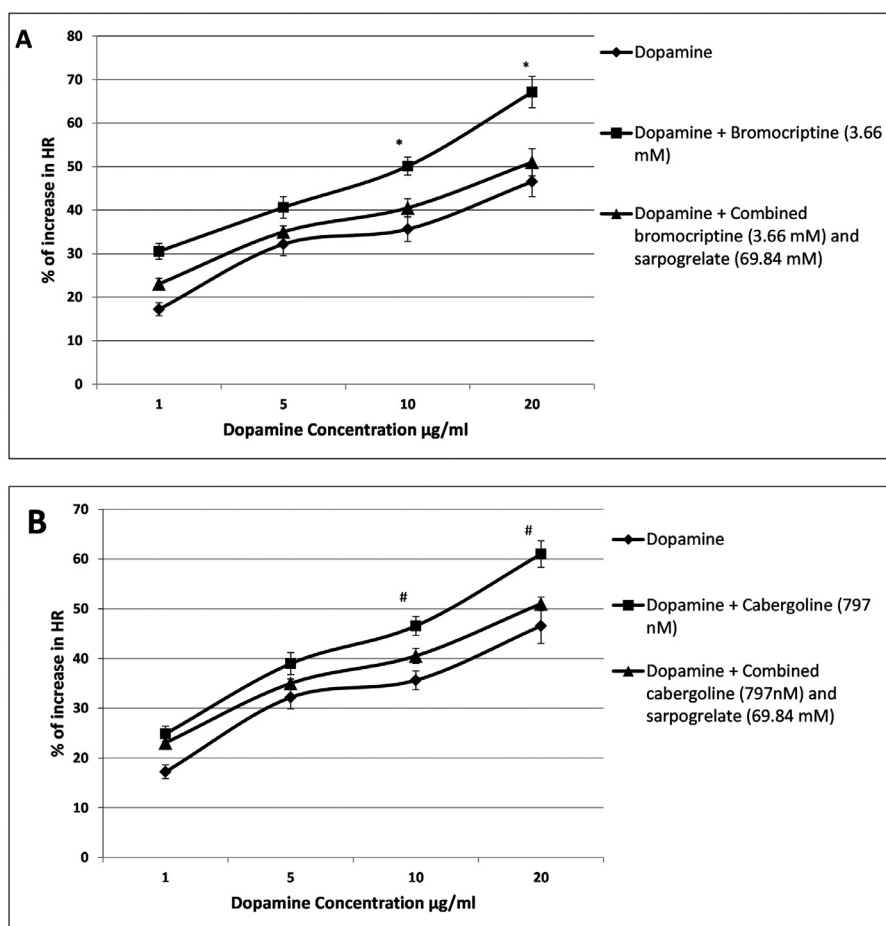
Na <sup>+</sup> , K <sup>+</sup> -ATPase activity	
Group*	μmol inorganic phosphate released/mg protein/h†
Normal control group	114.8 (12.9)
Diabetic control group	48.50 (3.62)‡
Diabetic group treated with bromocriptine	83.23 (8.69)§
Diabetic group treated with cabergoline	51.55 (4.97)
Diabetic group treated with sarpogrelate	54.75 (2.20)
Diabetic group treated with bromocriptine + sarpogrelate	78.71 (7.91)§
Diabetic group treated with cabergoline + sarpogrelate	55.27 (6.10)

\* n = 6 rats per group.

† Values are presented as mean (SEM).

‡ P < 0.05, significantly different from normal control group.

§ P < 0.05, significantly different from diabetic control group treated.



**Figure 2.** Panel A shows the dose–response curve of dopamine alone and in combination with the tested drugs (bromocriptine and sarpogrelate) on the isolated heart rate, individually. Panel B shows the dose–response curve of dopamine alone and in combination with the tested drugs (cabergoline and sarpogrelate) on the isolated heart rate. Values are presented as mean (SEM). n = 4 hearts per group. \*P < 0.05, significantly different from dopamine used.

to bromocriptine or cabergoline had significantly higher risk of developing both mild and moderate-severe regurgitation.<sup>41,42</sup> Sarpogrelate shows much more binding to the 5-HT<sub>2A</sub> over the 5-HT<sub>2B</sub> receptors without any affinity to other serotonin 5-HT and adrenergic receptors.<sup>43,44</sup> Sarpogrelate is a well-tolerated drug, and is used along with low-dose aspirin to help prevent heart attack and stroke in people with heart problems. It may also be used to prevent heart attack or stroke after certain heart surgeries. The most-known adverse effects include shortness of breath, headache, dizziness, nausea, proteinuria, increased urinary blood urea nitrogen and creatinine levels, anemia, increased levels of aspartate

transaminase/alanine transaminase and bilirubin, thrombocytopenia, agranulocytosis, and nosebleed may occur, it is contraindicated in patients with active pathological bleeding such as peptic ulcer or intracranial haemorrhage. In general, what can be gained by using sarpogrelate is much more significant than what might be risked. Our research suggests the potential therapeutic role of sarpogrelate when administered with dopamine D<sub>2</sub> agonists, and the utility of bromocriptine and cabergoline for long-term treatment for type 2 diabetes.

In the current study, the reported antidiabetes activity of the 2 tested drugs, bromocriptine and cabergoline, may have been due to

**Table 4**  
Effect of dopamine and the tested drugs on isolated hearts of rats.

Baseline hemodynamic characteristics <sup>f</sup>		
Group <sup>a</sup>	Heart rate (beats/min)	Cardiac contractility (%)
None preconditioned	174 (18)	
Dopamine 1 $\mu$ L	204 (14)	+15.34 (1.44) <sup>‡</sup>
Dopamine 5 $\mu$ L	230 (16)	+15.23 (1.23) <sup>‡</sup>
Dopamine 10 $\mu$ L	236 (13)	+19.23 (2.31) <sup>‡</sup>
Dopamine 20 $\mu$ L	255 (12)	+23.12 (1.65) <sup>‡</sup>
Bromocriptine 3.66 mM	194 (17)	+12.33 (1.42) <sup>‡</sup>
Cabergoline 797 nM	186 (11)	+11.23 (1.53) <sup>‡</sup>
Sarpogrelate 69.84 mM	161 (12)	No action

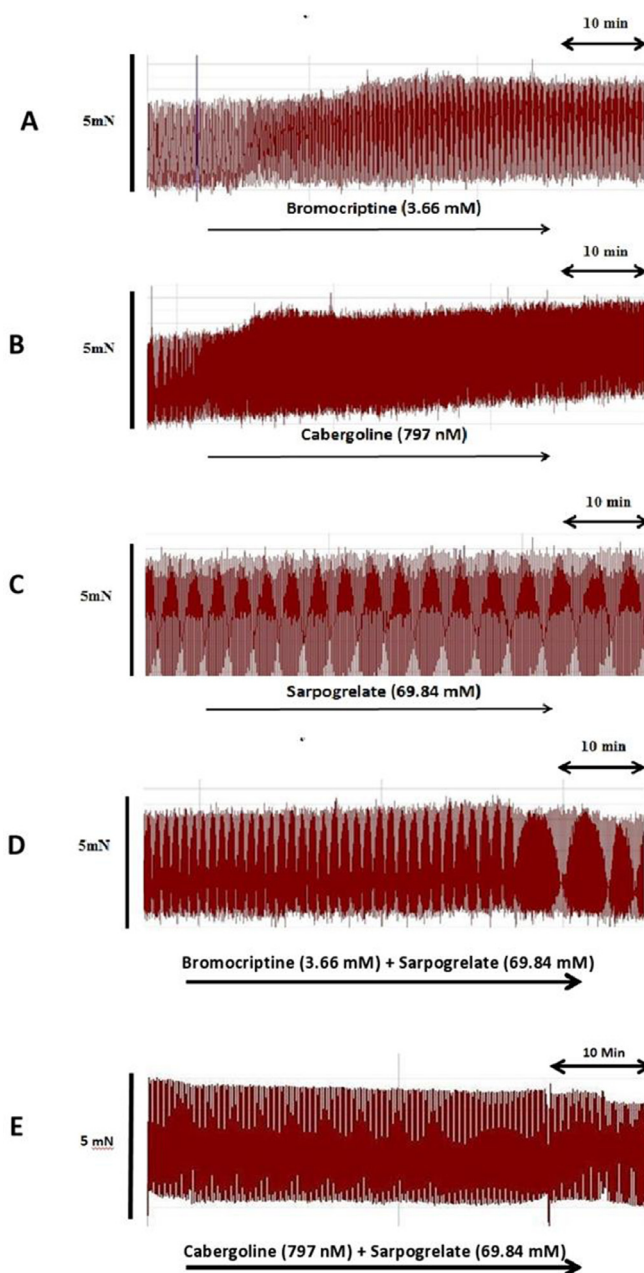
<sup>a</sup> Values are presented as mean (SEM).

<sup>f</sup> n = 4 hearts per group.

<sup>‡</sup> P < 0.05, significantly different from the none preconditioned group.

its dopaminergic D<sub>2</sub> activity centrally, which enhanced the pathway to stop the production of glucose in the liver. The data we found on antidiabetes activity is in agreement with some previous reported research.<sup>9,54</sup> The exact mechanism of their action as antidiabetes substances was not entirely identified. The previous studies illustrated some of the possible mechanisms of bromocriptine, including increases the production of the glucose transporter Glucose transporter 2 (GLUT2), and increases or mimics glucagon-like peptide-1 activity.<sup>45</sup> The reported antidiabetes activity may be related to its modulation action on the neurotransmitter centrally followed by improvement in the glucose tolerance and reduction of insulin resistance in diabetic rats.<sup>46</sup> Also the recorded inhibitory action of dopamine D<sub>2</sub> agonist drugs on serotonin pathway centrally may influence the rate of metabolism and profile of blood glucose levels.<sup>47</sup> A previous study illustrated a shifting in the level of serotonin throughout the day when using bromocriptine; this resets the circadian rhythms of the hypothalamus that control the monoamine neuronal activities that may responsible for improvement in the glucose tolerance and antagonizing the gluconeogenesis process.<sup>48</sup> Because of these previous reports, "bromocriptine mesylate was approved by the Food and Drug Administration (FDA) in May 2009 for the treatment of diabetes type 2."<sup>49</sup> In the current study of the tested drugs on the isolated islets, there was no direct action of bromocriptine or capergoline on insulin secretion compared with 11.8 mM glucose control. The results illustrated that the hypoglycemic activity of bromocriptine is not related to any activation on the pancreatic islets. Bromocriptine or cabergoline have not been shown to influence the secretion of insulin, but the action may be due to lowering hypothalamic dopamine levels and decreasing the activity of the sympathetic tone centrally, which reflects a decrease in the production of glucose from the liver, resulting in a decrease in postmeal blood glucose levels.<sup>9</sup> Bromocriptine does not have a specific receptor to mediate a direct response on glucose metabolism, but it acts by resetting sympathetic tone and the dopaminergic pathway centrally. These effects reduce post-prandial plasma glucose levels by suppressing hepatic glucose production and gluconeogenesis, and there is no reported action on pancreas or beta cells, which were already partial degenerated by the alloxan used in our research. Also, there are some studies stating that bromocriptine lowers plasma insulin levels,<sup>50,51</sup> and this action is opposite to all oral hypoglycemic drugs. This is why it is the only antidiabetes drug that shows no risk of hypoglycemia. Reduction of glucose and insulin levels as well as impaired lipid levels is the proposed mechanism of action of bromocriptine to improve glucose tolerance. However, the molecular mechanisms of action by which bromocriptine mediates its pharmacological effect exactly needs more investigation.

Diabetic nephropathy is the most common cause of different renal complications and hypertension. The 2 tested drugs, bromocriptine and cabergoline, showed antihypertension activity



**Figure 3.** Typical original recording of isolated heart of rat. The ordinate indicates force of contraction in milli-Newtons (mN), and the abscissae indicate time in minutes exemplified by scale bars. Panels A, B, and C show the effect of bromocriptine, cabergoline, and sarpogrelate individually. Panels D and E represent the effect of both bromocriptine and cabergoline in combination with sarpogrelate. Combination between dopamine D<sub>2</sub> agonists' bromocriptine and cabergoline with sarpogrelate show a marked decrease in its actions on the isolated heart.

as reported in some previous reports.<sup>52–54</sup> The mechanism of the antihypertension activity is related to its dopaminergic D<sub>2</sub> receptors activity, which deactivates the sympathetic action and modulates renal Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. "Bromocriptine and cabergoline induced a marked renal improvement by decreasing urea and creatinine serum levels in a model of diabetic nephropathy," as reported previously.<sup>54</sup> The mechanism pathways that cause this pharmacological action are not clearly stated. In the current study, Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was measured in the cortical homogenates of the kidneys in the diabetic groups. The decreased kidney Na<sup>+</sup>/K<sup>+</sup>-ATPase activity is a possible indication of an abnormality because it may affect the physiological and biochemical

functioning of the kidney with severe progress to nephropathy.<sup>55</sup> According to some previous reports,<sup>56,57</sup> the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase in models of renal ischemic cortex was markedly decreased relative to the control healthy renal cortexes. However, the current results conflict with some alternative studies that reported significant a rise or no change in the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase during kidney injury.<sup>58,59</sup> This disagreement may be related to the technique we used; for example, using the whole kidney instead of only the renal cortex for estimating the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase or due to the difference in models of diabetic nephropathy used in the studies. Nevertheless, pretreatment of the animals with bromocriptine in our experiments prevented the loss of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in diabetic nephropathy. Some of the previous studies showed that the drug bromocriptine stimulates Na<sup>+</sup>/K<sup>+</sup>-ATPase activity through its dopaminergic D<sub>2</sub> activity, which activates the phosphorylation pathway of p44/42 mitogen-activated protein kinases that play an important, recognized role in the determination of cell survival and activity in the kidney.<sup>60,61</sup> These reports illustrate the possible link between the activation of dopamine D<sub>2</sub> receptors done by bromocriptine and its role in resetting the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase in renal injury. The current result is in harmony with previous reports indicating “the potential role of dopamine D<sub>2</sub> agonists directed to increase renal D<sub>2</sub> receptor expression and its role for treatment of different forms of kidney diseases.”<sup>62</sup>

In the present study, a severe adverse effect of bromocriptine and cabergoline on the progress of myocardial injury was reported; the rats treated with bromocriptine and cabergoline for 1 month at doses of 10 mg/kg and 0.6 mg/kg, respectively, displayed significant elevation of CKM and troponin I in the serum. Also, there are some reported data about increased lactate dehydrogenase-1 and tumor necrosis factor  $\alpha$ -1 serum levels, and an identified marked area of infarcted myocardial muscles, observed by triphenyltetrazolium chloride staining, as a result of using bromocriptine and cabergoline.<sup>54</sup> The action may be related to the agonistic properties of these 2 drugs on serotonin 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors, increasing the heart rate. It was reported that both “bromocriptine and cabergoline have been associated with heart disease since the two drugs have both dopamine D<sub>2</sub> and serotonin 5-HT<sub>2A</sub> receptors agonistic properties.”<sup>17,18</sup> It was hypothesized that activation of serotonin 5-HT<sub>2A</sub> receptors would be among the main factors responsible for coronary artery vasoconstriction and initiation of different forms of heart disease.<sup>63</sup> These facts lead to the consideration that the drug sarpogrelate, a selective 5-HT<sub>2A</sub> receptor antagonist, may increase coronary blood flow and improve heart functions via alleviation of coronary vasoconstriction.<sup>64</sup> The results of the current study illustrate the benefits of using a combination of bromocriptine and cabergoline with sarpogrelate (selective 5-HT<sub>2A/2B</sub> antagonists) and the role of sarpogrelate in decreasing the adverse effects of these 2 drugs on the heart by its ability to decrease the serum levels of myocardial biomarkers like CK-MB and troponin I. There are previous reported data supporting the protective role of sarpogrelate on myocardial tissue by decreasing the serum levels of lactate dehydrogenase-1, and tumor necrosis factor  $\alpha$ -1 during the treatment of bromocriptine and cabergoline, and the ability of sarpogrelate to attenuate the identified infarct size in the myocardial tissues slices stained by triphenyltetrazolium chloride.<sup>54</sup> These results support some of the theories that serotonin and 5-HT<sub>2A</sub> receptors may play an important role in the development of myocardial injury-related diseases.

Dopamine has been reported to produce positive inotropic responses in isolated rat heart rat models.<sup>39,65</sup> Our findings demonstrate that dopamine also produces positive inotropic responses in rat hearts. The low concentrations of dopamine (< 10 M) reported in most of the previous studies act mainly on the dopaminergic receptors, whereas high concentrations of dopamine (> 10

M) can stimulate both dopaminergic and adrenergic  $\beta$  and  $\alpha$  receptors.<sup>66,67</sup> Dopaminergic receptors are cell-surface receptors coupled with G-proteins and classified into 2 main families. Regarding myocardial tissues, the D<sub>1</sub> receptors family includes D<sub>1</sub>, and D<sub>5</sub> receptor subtypes, which are reported mostly to be rare in myocardial tissue,<sup>68</sup> whereas the dopamine D<sub>2</sub>-like receptor family includes D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> receptor subtypes, which are linked to inhibition of adenylate cyclase, identified as functionally active receptors in the heart.<sup>69,70</sup> The pharmacologic action of dopamine on the heart are performed through dopaminergic receptors or through  $\beta$ <sub>1</sub> receptors; isolated hearts perfused with low concentrations of dopamine (eg, 1  $\mu$ M dopamine) showed relatively low pharmacologic action comparable to that observed in higher concentrations. Dopamine shows a high affinity to dopaminergic D<sub>2</sub>-like family receptors even at low concentrations.<sup>71</sup> Recently, a previous study illustrated that serotonin 5-HT<sub>2A</sub> receptors are widely expressed in the heart and interpose a hypertrophic response to serotonin in myocardial tissues.<sup>72</sup> This concept was confirmed in the current study when bromocriptine or cabergoline, which is a dopamine D<sub>2</sub> and 5-HT<sub>2A</sub> receptors agonists, were used.<sup>73</sup> Treatment with bromocriptine or cabergoline in combination with dopamine on an isolated heart showed a higher dose–response elevation in heart rate and force of contraction compared with the action of dopamine alone (Figures 2A and 2B). Using of sarpogrelate in combination with bromocriptine and cabergoline in presence of dopamine attenuated the percentage of the dose–response curve of heart rate in addition to decreasing the cardiac contractility and the role of sarpogrelate in decreasing both heart rate and force of contraction. The potential mechanism of these dopaminergic agonist drugs was shown to have affinity to the 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors.<sup>74</sup> A previous report stated partial agonistic activity of bromocriptine on 5-HT<sub>2A</sub> receptors coupled to cytosolic inositol phosphates, bromocriptine and cabergoline, by the same way activated phospholipase C, consistent with its partial affinity for 5-HT<sub>2A</sub>.<sup>75,76</sup> These in vitro results on isolated hearts support our in vivo results and confirm the benefits of using sarpogrelate (selective 5-HT<sub>2A</sub> or 5-HT<sub>2B</sub> antagonists) in combination with bromocriptine or cabergoline on myocardial function.

In the current research using parallel groups trials, the study *P* value is small, and the confidence interval for the effect did not include zero, and the study thus demonstrated a difference likely exists between the 2 groups. We concluded that the sample size was clearly enough to find a difference between the groups and the study clearly had adequate power to detect a difference. The findings of this study have to be seen in light of some limitations. There are 2 major limitations in this study that could be addressed in future research. First, bromocriptine (4 mg/kg) was tested in this study, according to guidelines from the American Diabetes Association; the maximum approved daily dose for human of bromocriptine is 4.8 mg. The dose of bromocriptine in this study seems higher than the normal dose. We used a relatively high dose—10 mg/kg—for enhancement the adverse effect of bromocriptine on myocardial tissue within 1 month, and the dose agrees with previous studies. Second, the study focused on the antidiabetes activity of the tested drugs, although they are widely used in treatment of hyperprolactinemia and Parkinson disease. More studies of different levels of drugs doses need to be used in different models of diseases in the future.

## Conclusions

According to our study, the dopamine D<sub>2</sub> receptor agonists bromocriptine and cabergoline induced myocardial infarction. However, using sarpogrelate in combination with bromocriptine or cabergoline decreased their potential adverse effects on myocardial



tissues. Thus, we suggest sarpogrelate deserves additional experimental and clinical research related to cardiovascular diseases.

## Acknowledgments

This project was funded by Batterjee Medical College, Jeddah, Saudi Arabia, project No. 2019-0020.

All authors conceived and planned the experiments. M. Fouad carried out the implementation of the experiments, planned and carried out the statistical analyses and contributed to sample preparation, and took the lead in writing the manuscript. H. Abd El-Latif, M. El-Yamani, M. Galal, S. Kamal, and I. Sindi contributed to the interpretation of the results; provided critical feedback; and supervised and helped shape the research, analysis, and manuscript.

M. Fouad thanks Professor Hekma Abdul Latif for her continuous support of the research as well as her patience, motivation, and immense knowledge. M. Fouad also thanks the rest of the thesis committee, Assistant Professor May Galal and Assistant Professor Mohamed El-Yammani, for their insightful comments, and Dr Iklas Sindi, Batterjee Medical College, Jeddah, Kingdom of Saudi Arabia, for providing the opportunity to work on this project in the college laboratories.

## Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

## References

- Lahlou S, Duarte GP. Hypotensive action of bromocriptine in the DOCA-salt hypertensive rat: Contribution of spinal dopamine receptors. *Fundam Clin Pharmacol.* 1998;12:599–606.
- Luchsinger A, Grilli M, Velasco M. Metoclopramide, and domperidone block the antihypertensive effect of bromocriptine in hypertensive patients. *Am J Ther.* 1998;5:81–88.
- Narkar V, Kunduzova O, Hussain T, Cambon C, Parini A, Lokhandwala M. Dopamine D2-like receptor agonist bromocriptine protects against ischemia/reperfusion injury in rat kidney. *Kidney Int.* 2004;66:633–640.
- Mejía-Rodríguez O, Herrera-Abarca JE, Ceballos-Reyes G, Avila-Diaz M, Prado-Urbe C, Belio-Caro F, Salinas-González A, Vega-Gomez H, Alvarez-Aguilar C, Lindholm B, García-López E, Paniagua R. Cardiovascular and renal effects of bromocriptine in diabetic patients with stage 4 chronic kidney disease. *BioMed research international.* 2013;2013.
- Mejía-Rodríguez O, Herrera-Abarca JE, Ceballos-Reyes G, Avila-Diaz M, Prado-Urbe C, Belio-Caro F, Salinas-González A, Vega-Gomez H, Alvarez-Aguilar C, Lindholm B, García-López E, Paniagua R. Cardiovascular and renal effects of bromocriptine in diabetic patients with stage 4 chronic kidney disease. *Biomed Res Int.* 2013;2013.
- Narkar Vihang, Kunduzova Oksana, Hussain Tahir, Cambon Claudie, Parini Angelo, Lokhandwala Mustafa. Dopamine D2-like receptor agonist bromocriptine protects against ischemia/reperfusion injury in rat kidney. *Kidney International. Volume.* 2004;66(2):633–640.
- Perlini S, Palladini G, Ferrero I, et al. Sympathectomy or doxazosin, but not propranolol, blunt myocardial interstitial fibrosis in pressure-overload hypertrophy. *Hypertension.* 2005;46(5):1213–1218.
- Miranda JM, Prieto RE, Paniagua R, et al. Clinical significance of serum and urine prolactin levels in lupus glomerulonephritis. *Lupus.* 1998;7(6):387–391.
- Defronzo RA. Bromocriptine: a sympatholytic, d2-dopamine agonist for the treatment of type 2 diabetes. *Diabetes Care.* 2011;34:789–794.
- Shivaprasad C, Kalra S. Bromocriptine in type 2 diabetes mellitus. *Indian J Endocrinol Metab.* 2011;15:S17–S24.
- Tan LC, Ng KK, Au WL, RK Lee, Chan YH, Tan NC. Bromocriptine use and the risk of valvular heart disease. *Mov Disord.* 2009;24(3):344–349.
- Herring N, Szmigielski C, Becher H, et al. Valvular heart disease and the use of cabergoline for the treatment of prolactinoma. *Clin Endocrinol (OXF).* 2009;70(1):104–108.
- Mokhles M. Dopamine agonist and heart failure in patients with Parkinson's disease: a nested case-control study on multiple health care databases. *Proceedings of the European Society of Cardiology Congress;* 2010.
- Tan Louis CS, Ng Kenneth KC, Au Wing-Lok, Lee Raymond KK, Chan Yiong-Huak, Tan Nigel CK. Bromocriptine use and the risk of valvular heart disease. *J Mov Disord.* 2009;24:344–349.
- Bordet R. [Central dopamine receptors: General considerations (Part 1)]. *Rev Neurol (Paris).* 2004;160:862–870 [Article in French].
- Wakil A, Rigby AS, Clark AL, Kallvikbacka-Bennett A, Atkin SL. Low dose cabergoline for hyperprolactinaemia is not associated with clinically significant valvular heart disease. *Eur J Endocrinol.* 2008;159:R11–R14.
- Kelekwska A, Hübner H, Grmeiner P, Pertz HH. The bulky N6 substituent of cabergoline is responsible for agonism of this drug at 5-hydroxytryptamine 5-HT2A and 5-HT2B receptors and thus is a determinant of valvular heart disease. *J Pharmacol Exp Ther.* 2011;338:381–391.
- Oana F, Onozuka H, Tsuchioka A, et al. Function and expression differences between ergot and non-ergot dopamine D2 agonists on heart valve interstitial cells. *J Heart Valve Dis.* 2014;23:246–252.
- Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G. Valvular heart disease and the use of dopamine agonists for Parkinson's disease. *N Engl J Med.* 2007;356(1):39–46.
- Rothman RB, Baumann MH, et al. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation.* 2000;102(23):2836–2841.
- Roth BL. Drugs and valvular heart disease. *N Engl J Med.* 2007;356(1):6–9.
- Brasil D, Tamsah RM, Kumar K, Kumamoto H, Takeda N, Dhalla NS. Blockade of 5-HT(2A) receptors by sarpogrelate protects the heart against myocardial infarction in rats. *J Cardiovasc Pharmacol Ther.* 2002;7:53–59.
- Satomura K, Takase B, Hamabe A, Ashida K, Hosaka H, Ohsuzu F, Kurita A. Sarpogrelate, a specific 5HT2-receptor antagonist, improves the coronary microcirculation in coronary artery disease. *Clin Cardiol.* 2002;25:28–32.
- Shinohara Y, Nishimaru K, Sawada T, et al. Sarpogrelate-aspirin comparative clinical study for efficacy and safety in secondary prevention of cerebral infarction (S-ACCESS): a randomized, double-blind, aspirin-controlled trial. *Stroke.* 2008;39:1827–1833.
- Saini HK, Takeda N, Goyal RK, Kumamoto H, Arneja AS, Dhalla NS. Therapeutic potentials of sarpogrelate in cardiovascular disease. *Cardiovasc Drug Rev.* 2004;22:27–54.
- Pourghasem M, Nasiri E, Shafi H. Early renal histological changes in alloxan-induced diabetic rats. *Int J Mol Cell Med.* 2014;3(1):11–15.
- Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR J.* 2002;43:244–258.
- Ribeiro-de-Oliveira Jr A, Guerra RM, Fóscolo RB, Marubayashi U, Reis AM, Coimbra CC. Effects of chronic bromocriptine (CB-154) treatment on the plasma glucose and insulin secretion response to neurocytopenia in rats. *J Endocrinol.* 1999;162:237–242.
- Ibrahim N, Zidan RA, Karam R. Does green tea have an ameliorative effect against cabergoline-induced cardiotoxicity in adult male albino rats? A histological and biochemical study. *Egy J Hist.* 2012;35:13–22.
- Kim DH, Choi BH, Ku SK, Park JH, Oh E, Kwak MK. Beneficial effects of sarpogrelate and rosuvastatin in high fat diet/streptozotocin-induced nephropathy in mice. *PLoS One.* 2016;11(4) Published 2016 Apr 20.
- Mukherjee KI. Medical laboratory technology. *Tata McGraw Hill.* 1988;3:991–993.
- Younis NS, Abduldaium MS, Mohamed ME. Protective Effect of Geraniol on Oxidative, Inflammatory and Apoptotic Alterations in Isoproterenol-Induced Cardiotoxicity: Role of the Keap1/Nrf2/HO-1 and PI3K/Akt/mTOR Pathways. *Antioxidants (Basel).* 2020;9(10):977.
- Moreira JB, Wohlwend M, Alves MN, Wislöff U, Bye A. A small molecule activator of AKT does not reduce ischemic injury of the rat heart. *J Transl Med.* 2015;1:76 13.
- Li D, Peng SY, Zhang ZW, et al. Complete disassociation of adult pancreas into viable single cells through cold trypsin-EDTA digestion. *J Zhejiang Univ Sci B.* 2013;14(7):596–603.
- Chandravanshi B, Dhanushkodi A, Bhonde R. High Recovery of Functional Islets Stored at Low and Ultralow Temperatures. *Rev Diabet Stud.* 2014;11(3-4):267–278.
- Patil Swapnil B, Dongare Vandana R, Kulkarni Chaitanya R, Joglekar Madhav M, Arvindekar Akalpita U. Antidiabetic activity of *Kalanchoe pinnata* in streptozotocin-induced diabetic rats by glucose independent insulin secretagogue action. *Pharmaceutical Biology.* 2013;51:1411–1418 11.
- Beltowski J, Marciniak A, Wójcicka G, Górny D. The opposite effects of cyclic AMP-protein kinase a signal transduction pathway on renal cortical and medullary Na<sup>+</sup>,K<sup>+</sup>-ATPase activity. *J Physiol Pharmacol.* 2002 Jun;53(2): 211–231.
- Verma SC, McNeill JH. Action of imidazole on the cardiac inotropic phosphor-ylase activating and cyclic AMP producing effects of norepinephrine and histamine. *Res. Commun. Chem. Pathol. Pharmacol.* 1974;7:305–319.
- Lazou A, Markou T, Zioga M, Vasara E, Efstathiou A, Gaitanaki C. Dopamine mimics the cardioprotective effect of ischemic preconditioning via activation of alpha1-adrenoceptors in the isolated rat heart. *Physiol Res.* 2006;55(1):1–8.
- Curtis MJ, Bond RA, Spina D, Ahluwalia A, Alexander SP, Giembycz MA, Gilchrist A, Hoyer D, Insel PA, Izzo AA, Lawrence AJ, MacEwan DJ, Moon LD, Wonacott S, Weston AH, McGrath JC. Experimental design and analysis and their reporting: new guidance for publication in BJP [published correction appears in Br J Pharmacol. 2015 Sep; 172(18):4600]. *Br J Pharmacol.* 2015;172:3461–3471.
- Kim JY, Chung EJ, Park SW, Lee WY. Valvular heart disease in Parkinson's disease treated with ergot derivative dopamine agonists. *Mov Disord.* 2006;21(8):1261–1264.
- Junghanns S, Fuhrmann JT, Simonis G, Oelwein C, Koch R, Strasser RH, Reichmann H, Storch A. Valvular heart disease in Parkinson's disease patients treated with dopamine agonists: a reader-blinded monocenter echocardiography study. *Mov Disord.* 2007;22(2):234–238.

43. Muntasir HA, Hossain M, Bhuiyan MA, et al. Selectivity of sarpogrelate to the recombinant human 5-HT<sub>2A</sub> receptors as assessed by molecular pharmacology and molecular modeling. *Pharmacometrics*. 2007;72:39–49.
44. Israilova M, Suzuki F, Tanaka T, Nagatomo T, Taniguchi T, Muramatsu I. Binding and functional affinity of sarpogrelate its metabolite m-1 and ketanserin for human recombinant alpha-1-adrenoceptor subtypes. *Pharmacology*. 2002;65:69–73.
45. Scranton R, Cincotta A. Bromocriptine—unique formulation of a dopamine agonist for the treatment of type 2 diabetes. *Expert Opin Pharmacol*. 2010;11:269–279.
46. Aminorroaya A, Janghorbani M, Ramezani M, Haghighi S, Amini M. Does bromocriptine improve glycemic control of obese type-2 diabetics? *Horm Res*. 2004;62:55–59.
47. Shivaprasad C, Kalra S. Bromocriptine in type 2 diabetes mellitus. *Indian J Endocrinol Metab*. 2011;15(Suppl 1):S17–S24.
48. Luo S, Luo J, Cincotta AH. Association of the antidiabetic effects of bromocriptine with a shift in the daily rhythm of monoamine metabolism within the suprachiasmatic nuclei of the Syrian hamster. *Chronobiol Intern*. 2000;17(2):155–172.
49. Mahajan R. Bromocriptine mesylate: FDA-approved novel treatment for type-2 diabetes. *Indian J Pharmacol*. 2009;41(4):197–198.
50. Cincotta AH, Schiller BC, Meier AH. Bromocriptine inhibits the seasonally occurring obesity, hyperinsulinemia, insulin resistance, and impaired glucose tolerance in the Syrian hamster, *Mesocricetus auratus*. *Metabolism*. 1991;40(6):639–644.
51. Derkach KV, Ivantsov AO, Sukhov IB, et al. Restoration of hypothalamic signaling systems as a cause of improved metabolic parameters in rats with neonatal diabetes model during treatment with bromocriptine mesylate. *Cell Tiss. Biol*. 2017;11:234–241.
52. Zeng C, Zhang M, Asico LD, Eisner GM, Jose PA. The dopaminergic system in hypertension. *Clin Sci (Lond)*. 2007;112:583–597.
53. McCoy CE, Douglas FL, Goldberg LI. Selective antagonism of the hypotensive effects of dopamine agonists in spontaneously hypertensive rats. *Hypertension*. 1986;8:298–302.
54. Fouad M, Latif HAAE, Galal M, et al. Therapeutic activity of sarpogrelate, and dopamine D<sub>2</sub> receptor agonists on cardiovascular and renal systems in alloxan-induced diabetic rats. *Research Square*. 2020. doi:10.21203/rs.3.rs-44926/v2.
55. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacol Rev*. 2002;52:673–751.
56. KAJIWARA I, KAWAMURA K, HIRATSUKA Y, TAKEBAYASHI S. The influence of oxygen free radical scavengers on the reduction of membrane-bound Na<sup>+</sup>-K<sup>+</sup>-ATPase activity induced by ischemia/reperfusion injury in canine kidney. *Nephron*. 1996;72:637–643.
57. AYDIN S, ARICIOGLU A, TURKOZKAN N, et al. Na<sup>+</sup>,K<sup>+</sup>-ATPase activity of rabbit kidney cortex membranes in ischemia and reperfusion. *Acta Biochem Pol*. 1993;40:545–547.
58. Fekete A, Vannay A, Vér A, Vásárhelyi B, Müller V, Ouyang N, Reusz G, Tullassay T, Szabó AJ. Sex differences in the alterations of Na<sup>+</sup>(+), K<sup>+</sup>(+)-ATPase following ischaemia-reperfusion injury in the rat kidney. *J Physiol*. 2004;1(Pt 2):471–480 555.
59. WANG Z, RABB H, HAQ M. A possible molecular basis of natriuresis during ischemic-reperfusion injury in the kidney. *J Am Soc Nephrol*. 1998;9(4):605–613.
60. NARKAR VA, HUSSAIN T, PEDEMONTE C, et al. Dopamine D<sub>2</sub> receptor activation causes mitogenesis via p44/42 mitogen-activated protein kinase in opossum kidney cells. *J Am Soc Nephrol*. 2001;12:1844–1852.
61. NARKAR VA, HUSSAIN T. LOKHANDWALAMF: Role of tyrosine kinase and p44/42 MAPKs in D<sub>2</sub>-like receptor-mediated stimulation of Na<sup>+</sup>, K<sup>+</sup>, ATPase in kidney. *Am J Physiol: Renal Physiol*. 2002;282:F697–F702.
62. Konkalmatt PR, Asico LD, Zhang Y, Yang Y, Drachenberg C, Zheng X, Han F, Jose PA, Armando I. Renal rescue of dopamine D<sub>2</sub> receptor function reverses renal injury and high blood pressure. *JCI Insight*. 2016;1:e85888.
63. Nichols CD. Serotonin 5-HT<sub>2A</sub> Receptor function as a contributing factor to both neuropsychiatric and cardiovascular diseases. *Cardiovasc Psychiatry Neurol*. 2009;2009.
64. Fujita M, Minamino T, Sanada S, Asanuma H, Hirata A, Ogita H, Okada K, Tsukamoto O, Takashima S, Tomoike H, Node K, Hori M, Kitakaze M. Selective blockade of serotonin 5-HT<sub>2A</sub> receptor increases coronary blood flow via augmented cardiac nitric oxide release through 5-HT<sub>1B</sub> receptor in hypoperfused canine hearts. *J Mol Cell Cardiol*. 2004;37:1219–1223.
65. HOLMES JC, FOWLER NO. Direct cardiac effects of dopamine. *Circ Res*. 1962 Jan;10:68–72.
66. EMILIE G, MALOTEUX JM, GEURTS M, HOOGENBERG K, CRAGG S. Dopamine receptors – physiological understanding to therapeutic intervention potential. *Pharmacol Ther*. 1999;84:133–156.
67. MURPHY MB. Dopamine: a role in the pathogenesis and treatment of hypertension. *J Hum Hypertens*. 2000;14:S47–S50.
68. Ozono R, O'Connell DP, Vaughan C, Botkin SJ, Walk SF, Felder RA, Carey RM. Expression of the subtype 1A dopamine receptor in the rat heart. *Hypertension*. 1996;27:693–703.
69. Gómez Mde J, Rousseau G, Nadeau R, Berra R, Flores G, Suárez J. Functional and autoradiographic characterization of dopamine D<sub>2</sub>-like receptors in the guinea pig heart. *Can J Physiol Pharmacol*. 2002;80(6):578–587.
70. Cavallotti C, Nuti F, Bruzzone P, Mancone M. Age-related changes in dopamine D<sub>2</sub> receptors in rat heart and coronary vessels. *Clin Exp Pharmacol Physiol*. 2002;29(5-6):412–418.
71. Seeman P, Van Tol HH. Dopamine receptor pharmacology. *Trends Pharmacol Sci*. 1994;15(7):264–270.
72. Lairez O, Cognet T, Schaak S, Calise D, Guilbeau-Frugier C, Parini A, Miallet-Perez J. Role of serotonin 5-HT<sub>2A</sub> receptors in the development of cardiac hypertrophy in response to aortic constriction in mice. *J Neural Transm (Vienna)*. 2013;120(6):927–935.
73. Newman-Tancredi A, Cussac D, Quentric Y, Touzard M, Verrière L, Carpentier N, Millan MJ. Differential actions of anti-Parkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT<sub>1</sub> and 5-HT<sub>2</sub>, receptor subtypes. *J Pharmacol Exp Ther*. 2002;303(2):815–822.
74. Valassi Elena, Klibanski Anne, Biller Beverly MK. Potential Cardiac Valve Effects of Dopamine Agonists in Hyperprolactinemia. *J Clin End & Metab*. 2010;95(3):1025–1033.
75. Mitchell A, Phipps S, Smith D, Elliott J. Bromocriptine acts as a partial agonist at the humans 5-HT<sub>2A</sub> receptor. *Br J Pharmacol*. 1998;124:60.
76. Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned, recombinant, human receptor subtypes. *J Pharmacol Exp Ther*. 2002;303:791–804.