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Research Article

Cardiovascular and Renal Effects of Dopamine Receptor D_2 Acting Drugs in Alloxan-Induced Diabetic Rats

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Abstract

Background and Purpose: Bromocriptine and cabergoline are well-established drugs in Parkinsonism, hyperprolactinaemia and antihyperglycemia. An association has been demonstrated between valvular heart disease and long-term treatment with dopamine agonist use in patients treated for Parkinson's disease and hyperprolactinemic.

Experimental Approach: The present study aims to examine the effect of sarpogrelate, a 5-HT (2A) receptor blocker, in decreasing myocardial injury (MI) induced by long term use of D2 agonist drugs in diabetic rats. Daily dose of bromocriptine (4 mg/kg IP) and cabergoline (0.6 mg/kg IP) were administered individually and in combination with sarpogrelate or domperidone to diabetic rats for 4 weeks.

Key Results: Both of bromocriptine and cabergoline showed a significant decrease in BGL, BP and kidney hypertrophy index in diabetic nephropathy rats. Daily oral administration of bromocriptine, cabergoline even alone or in combination with sarpogrelate caused significant decreased in serum concentrations of AST, ALP, urea and creatinine. Bromocriptine and cabergoline displayed significant elevation of LDH-1, Troponin I and TNFα1 levels in the serum ($p < 0.05$). By contrast, Using combination of bromocriptine and cabergoline with sarpogrelate treatment significantly decreased the myocardial biomarkers level in the serum. Percentage of myocardial injury size in the heart was evaluated using TTC staining method. Combination of bromocriptine or cabergoline with sarpogrelate (50 mg/kg) reduced the percentage of the myocardial infarct size.

Conclusion and Implications: The study demonstrated that both of bromocriptine and cabergoline can be used safely in combination with sarpogrelate for treatment of many diseases like hypertension, diabetes and Parkinsonism.

Keywords: *Bromocriptine; Cabergoline; Sarpogrelate; Diabetic Nephropathy; Myocardial Injury; TNFα1*

Abbreviations

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; TNF: Tumor Necrosis Factor; TTC: Triphenyl Tetrazolium Chloride; LDH: Lactate Dehydrogenas.

Introduction

Among to the various types of diabetes complications, diabetic nephropathy is the most common renal complication and the leading cause of end-stage renal disease. During the later stages of diabetic nephropathy, transforming growth factor-ß1 overexpression, extracellular matrix deposition, and loss of glomerular architecture define glomerulosclerosis [1]. Although the pathogenesis of diabetic nephropathy is complex, hyperglycemia is the primary factor that underlies the initiation of diabetic nephropathy [2]. It has been demonstrated in several *in vitro* studies of STZ-induced diabetic nephropathy where high glucose-induced renal damage is associated with excessive production of reactive oxygen species (ROS) under hyperglycemic conditions [3-5].

Bromocriptine is a sympatholytic D_2-dopamine agonist that has been approved for the treatment of type 2 diabetes. Based on animal and human studies, timed bromocriptine administration within 2 h of awakening is believed to augment low hypothalamic dopamine levels and inhibit excessive sympathetic tone within the central nervous system, resulting in a reduction in post-meal plasma glucose levels due to enhanced suppression of hepatic glucose production [6]. Agonists acting on dopamine D_2 receptors can lower blood pressure by vasodilation, inhibition Na/k ATPase activity and inhibition of sympathetic nerve activity. Bromocriptine (D_2 agonist) may protect against I/R injury of the kidney via p44/42 mitogen-activated protein kinase activation [7], and also prevents the progression of chronic kidney disease [8]. Domperidone (D_2 antagonist) partially reduced the hypotensive effect of bromocriptine. In contrast, the bromocriptine-induced hypotension was fully abolished by pretreatment with metoclopramide, a dopamine D_2 receptor antagonist that crosses the blood-brain barrier [9,10].

However, use of D_2 agonists might be associated with cardiovascular complications including orthostatic hypotension and heart failure. There is a direct relationship between the use of D_2-like R agonist in patients with Parkinson's disease and heart failure especially in early phase of therapy [11]. Dopamine D_2 receptor agonists are classified as ergot dopamine D_2 agonists and non-ergot D_2 agonists. Bromocriptine and cabergoline (ergot derivative) have been associated with valvular heart disease, since the two drugs have both potent D_2 receptor and serotonin 5-HT (2B) receptor agonistic properties. Pramipexole (non-ergot derivative) has few incidences of heart valve disease onset, since it has no effect on 5-HT (2B) receptors [12-14].

Sarpogrelate and ketanserin (selective 5HT2a/2b antagonists) attenuates cardiac dysfunction, infarct size, and changes in the ECG due to MI. These results also support the view that serotonin and 5-HT (2A) may contribute to the deleterious effects of ischemic injury in the heart [15,16]. Sarpogrelate has been found to have beneficial effects in peripheral vascular disease, restenosis after coronary stenting, pulmonary hypertension, acute and chronic myocardial infarction. The present study aims to examine the protective effect of sarpogrelate, a 5-HT (2A) receptor blocker, with long term use of D_2 agonist drugs on cardiovascular functions in diabetic nephropathy model of rats.

Materials and Methods

Materials

Sarpogrelate was obtained from Shanghai Linebon Ltd. Shanghai, China. Bromocriptine was from Novartis, Italy. Cabergoline was from Pfizer, Italy. Domperidone was from Jamjoom pharmaceutical company, KSA. Alloxan was obtained from Sigma-Aldrich (St Louis, MO, USA). Urea Assay Kit, Creatinine Assay Kit, Alkaline Phosphatase Assay Kit, Aspartate Aminotransferase Activity Assay Kit, LDH-1 assay kit and TNF alpha ELISA Kit were obtained from Abcam. Troponin I test kit was from Encode Medical Engineering Company. 2,3,5-Triphenyltetrazolium chloride was from Gold Biotechnology, USA. All other reagents used were of the highest grade commercially available.

Animals

Male Wister albino rats were housed three per cage under standard laboratory conditions in ventilated cages and given food and water ad libitum. As some suffering might result from these experiments, the Batterjee medical college committee for Research and Ethical Guidelines were followed. Great care was taken, particularly with regard to housing conditions, to avoid or minimize discomfort of the

animals. The animals were kept on solid floored cages with a deep layer of sawdust to accommodate the excess of urination and cages were changed daily. All animals were euthanized by thiopental (intravenous injection, 150 mg/kg) for tissue collection.

Induction of diabetes by alloxan

Alloxan monohydrate was dissolved in sterile normal saline. Diabetes was induced in 30 rats (150 - 200g) by a single intraperitoneal injection of alloxan (5%) 150 mg/kg. The rats were kept fasting for 12h before and after Alloxan injection. Fasting plasma glucose was measured by obtaining blood samples from the tails of animals. The rats which showed plasma glucose level of 200 mg/dL or more were considered diabetic [17].

Experimental design

Tests took place 4 weeks after the induction of diabetes. The rats were divided into 8 groups, each group of 6 rats. Once there was a stable elevation in the urea and creatinine in the blood of the diabetic rats, drugs were injected:

1. Normal control group (Saline, IP).
2. Diabetic control group (Saline, IP).
3. Diabetic group treated with bromocriptine (4 mg/kg, IP).
4. Diabetic group treated with cabergoline (0.6 mg/kg, IP).
5. Diabetic group treated with domperidone (10 mg/kg, IP).
6. Diabetic group treated with sarpogrelate (50 mg/kg, IP).
7. Diabetic group treated with combination between bromocriptine and sarpogrelate by same doses.
8. Diabetic group treated with combination between cabergoline and sarpogrelate by same doses.
9. Diabetic group treated with combination between cabergoline and domperidone by same doses.

Determination of blood glucose level

Blood glucose levels were tested on the 0 day, 1st, 7th, 14th and 21st days from the start of the experiment. Blood samples were collected from the tail of the fasting animals. One millimeter of its end was cut and a drop of blood was used for blood glucose test using advanced glucometer (Roche, USA). The accuracy of glucometer was checked with O-toluidine method [18].

Blood pressure recording

Basal blood pressure was measured using non-invasive blood pressure recorder apparatus (Ugo basile instruments, Italy). Each rat was placed in restrainer and appropriate cuff with sensor was mounted on its tail and warmed to about 33 - 35 °C. The tail cuff was inflated to a pressure above 200 mmHg, systolic blood pressure; diastolic blood pressure was measured directly by the tail cuff and pulse sensor two hours after treatment of drugs [19].

Estimation of liver and kidney functions

Alkaline phosphatase and aspartate aminotransferase activities were determined using the method as described by King and King [20]. The procedure of Tietz., *et al.* [21], was used to determine serum creatinine concentration while the serum urea concentration was

determined by the method of Kaplan [22]. After ending of the study, the kidneys were washed with saline and weighed. The kidney weight and body weight ratio (g/g) x 10^3 were calculated for each rat in order to determine the kidney hypertrophy index.

Estimation of serum biomarkers of myocardial injury

The blood was withdrawn by retro-orbital venous plexus, kept at 37°C for 30 min, and centrifuged at 4°C, 3000 g for 10 min. Then the separated serum was stored at -20°C for various biochemical analyses. The severity of cardiac injury was assessed by the estimation of lactate dehydrogenase (LDH-1) and cardiac troponin I (cTnI) in serum. LDH-1 and troponin levels were analyzed by spectrophotometric methods using commercially available diagnostic kits according to the methods of Nieland [23].

Serum TNF-α1 concentrations

Serum TNF-alpha cytokines level were identified by ELISA technique using a quantitative sandwich enzyme immunoassay technique (EASIA kits for TNF-α by Abcam Company). All tests were done according to company's instruction. The results calculated by ELISA reader (optical density at 405nm immediately) and applied on a standard curve in order to sort out the cytokines concentration.

Evaluation of myocardial infarct size by TTC

To evaluate tissue death, the hearts were removed and washed in phosphate buffered saline, frozen and stored at -20°C for 30 minutes and sliced into 1 mm sections perpendicularly along the long axis from apex to base.

Triphenyl tetrazolium chloride (TTC) staining was used to assess myocardial tissue viability and determine myocardial infarct size. The tissue slices were incubated in 1% TTC PBS solution, pH 7.4, at 37°C for 20 minutes. Tissues were fixed in 10% PBS-buffered formalin overnight at 2°C - 8°C. Both sides of each TTC-stained tissue slice were photographed with the digital camera to distinguish the red stained viable and the white-unstained necrotic tissues [24].

The digital photographs were downloaded to a personal computer. Areas stained in red and white were measured using SigmaScan software (SPSS Science) in trace-measurement mode. That mode was used to measure either the ischemic area or the infarcted area, which is a sum of calibrated pixels in a defined region, through manually drawing an image layer on the photograph [25]. The infarction size percentage was calculated by the following equation:

% Infarct volume = Infarct volume/Total volume of slice × 100.

Statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology [26]. The results are expressed as mean± SE. The significance of the differences between the values was performed by one-way ANOVA test and Dunnett's Multiple Comparison Test using GraphPad Prism software. $P < 0.05$ was considered to be a significant difference.

Results and Discussion

Estimation of blood glucose levels

The diabetic control group showed a significant rise in blood glucose level as compared to the normal control Group. On repeated administration of the bromocriptine (4 mg/kg), cabergoline (0.6 mg/kg) individually or in combination with sarpogrelate or domperidone, a significant ($p < 0.05$) decrease in blood glucose by time as compared to the diabetic control group (Table 1). Administration of domperidone alone induced a significant decrease in the BGL as compared to the diabetic control group. Both of bromocriptine and cabergoline induced antihyperglycemia activity which may be due to enhanced suppression of hepatic glucose production [6].

BGL mg/dL				
Groups	**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 ± 45.13*	328 ± 141.34*	428.5 ± 121.17*	396.5 ± 80.60*
Diabetic group treated with Bromocriptine	191 ± 16.89#	199.25 ± 65.05#	238.25 ± 56.88#	284.25 ± 148.00#
Diabetic group treated with Cabergoline	213.75 ± 37.74#	211.75 ± 38.62#	293 ± 213.15#	280.25 ± 220.48#
Diabetic group treated with Domperidone	230.25 ± 45.52#	184.75 ± 13.5#	213.15 ± 134.34#	198.25 ± 121.34#
Diabetic group treated with Sarpogrelate	326.25 ± 93.45	298.25 ± 122.37	393.75 ± 118.77	369.75 ± 43.15
Diabetic group treated with Bromocriptine +sarpogrelate	247.75 ± 50.35#	215.5 ± 33.20#	310.25 ± 70.59#	288.25 ± 109.41#
Diabetic group treated with Cabergoline + sarpogrelate	196 ± 54.20#	158.75 ± 35.01#	235.5 ± 182.22#	215.75 ± 141.46#
Diabetic group treated with Cabergoline + domperidone	253.75 ± 42.75#	250 ± 28.84#	147.33 ± 40.27#	125.33 ± 32.25#

Table 1: *Effect of the tested drugs on BGL in alloxan-induced diabetic rats. Values shown are means ± SEM; n = 5 rats per group. *: P < 0.05, significantly different from normal control group; #: P < 0.05, significantly different from diabetic control group.*

Kidney hypertrophy index

The results demonstrated that the kidney hypertrophy index was significantly increased in the diabetic group of rats compared with the normal control rats. However, the index was found to be markedly reduced by both of bromocriptine and cabergoline treated groups even individually or mixed with sarpogrelate (Table 2). There is no effect of using sarpogrelate or domperidone on diabetic kidney index. Combination of cabergoline with domperidone didn't alter its protection effect on kidney index.

Kidney Hypertrophic Index	
Groups	**KHI (g/g * 1000)**
Normal control group	4.555 ± 0.423
Diabetic control group	6.6125 ± 0.57*
Diabetic group treated with Bromocriptine	4.7475 ± 0.33#
Diabetic group treated with Cabergoline	4.81 ± 0.49#
Diabetic group treated with Domperidone	6.07 ± 0.21
Diabetic group treated with Sarpogrelate	5.90 ± 0.27
Diabetic group treated with Bromocriptine +sarpogrelate	4.715 ± 0.61#
Diabetic group treated with Cabergoline + sarpogrelate	4.46 ± 0.48#
Diabetic group treated with Cabergoline + domperidone	4.17 ± 0.88#

Table 2: *Effect of the tested drugs on kidney hypertrophic index in alloxan-induced diabetic rats. Values shown are means ± SEM; n = 5 rats per group. *: P < 0.05, significantly different from normal control group; #: P < 0.05, significantly different from diabetic control group.*

Hemodynamic parameter (Antihypertensive activity)

Alloxan-induced diabetes in rats caused significant rise in blood pressure after 3 weeks from induction of diabetes. Daily oral administration of bromocriptine and cabergoline individually or in combination with sarpogrelate showed a significant decrease in blood pressure (Table 3). There is no effect of sarpogrelate or domperidone on BP in diabetic rats. Combination of cabergoline with domperidone showed a marked increase in the BP at week 4. According to the present study, diabetic nephropathy is the most common renal complication and the leading cause of hypertension. Both of bromocriptine and cabergoline showed a marked antihypertensive activity and this agree with some previous report [27,28]. This action related to agonists acting on dopamine D_2 receptors can lower blood pressure by vasodilatation, inhibition Na/k ATPase activity and inhibition of sympathetic nerve activity.

Mean Blood pressure MBP (mmHg)				
Groups	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Normal control group	110 ± 4.01	118.25 ± 8.99	108.75 ± 4.57	111.5 ± 4.20
Diabetic control group	112.5 ± 9.14	121.75 ± 11.35	150.75 ± 5.25*	151.25 ± 5.12*
Diabetic group treated with Bromocriptine	115 ± 9.12	102.75 ± 6.8	109 ± 10.23#	121.75 ± 15.26#
Diabetic group treated with Cabergoline	103.75 ± 7.0	105.5 ± 9.14	123 ± 7.87#	107.75 ± 107.75#
Diabetic group treated with Domperidone	130.5 ± 14.7	119 ± 10.80	139 ± 4.9	140.75 ±18.22
Diabetic group treated with Sarpogrelate	118.75 ± 10.87	114.75 ± 8.53	137.75 ± 8.53	139 ± 18.56
Diabetic group treated with Bromocriptine +sarpogrelate	104.5 ± 7.59	104.75 ± 4.99	106.25 ± 11.08#	118 ± 13.03#
Diabetic group treated with Cabergoline + sarpogrelate	109.25 ± 11.58	105 ± 9.05	106 ± 18.3#	104.25 ± 5.5#
Diabetic group treated with Cabergoline + domperidone	113.75 ± 6.0	120.5 ± 7.59	104.33 ± 22.05#	140.25 ± 9.42#

Table 3: *Effect of the tested drugs on mean blood pressure in alloxan-induced diabetic rats. Values shown are means ± SEM; n = 5 rats per group. *: P < 0.05, significantly different from normal control group; #: P < 0.05, significantly different from diabetic control group.*

Estimation of liver and kidney functions

Serum concentrations of urea, creatinine, AST and ALP as indicator of liver and kidney functions were recorded in the table 4. Data revealed that diabetic control group had significant increase in serum concentrations of urea, creatinine, AST and ALP compared to the normal control group. Daily ip administration of bromocriptine, cabergoline even alone or in combination with sarpogrelate caused significant decreased in serum concentrations of urea, creatinine, AST and ALP, compared to the positive diabetic group. Daily ip administration of domperidone showed significant decreased in serum concentrations of urea, creatinine, AST and ALP. There is no marked effect of sarpogrelate on all of the previous biochemical indicators in the diabetic rats. Both of bromocriptine and cabergoline induced a marked improvement in the function of the kidney in model of alloxan induced diabetic nephropathy in rats by decreasing serum levels of urea and creatinine.

Biochemicals				
Group	**ALP(IU/L)**	**AST(IU/L)**	**Urea (mmol/L)**	**Creatinine (mmol/L)**
Normal control group	60.85±8.77	25.04±4.96	43.38±4.77	0.46 ± 0.07
Diabetic control group	89.05±10.44*	45.86±7.57*	75.81±18.85*	1.89 ± 0.19*
Diabetic group treated with Bromocriptine	68.83±7.27#	34.18±5.78#	60.41±6.22#	1.04 ± 0.26#
Diabetic group treated with Cabergoline	73.27±7.93#	21.37±10.05#	52.09±2.23#	0.79 ± 0.11#
Diabetic group treated with Domperidone	66.38±5.38#	28.60±5.50#	56.43±5.82#	0.92 ± 0.09#
Diabetic group treated with Sarpogrelate	82.16±23.83	41.47±10.02	69.41±7.65	1.68 ± 0.25
Diabetic group treated with Bromocriptine +sarpogrelate	64.8±7.21#	31.03±6.44#	44.4±8.23#	1.26 ±0.25#
Diabetic group treated with Cabergoline + sarpogrelate	73.44±6.68#	33.79±11.18#	51.16±10.43#	1.33 ± 0.21#
Diabetic group treated with Cabergoline + domperidone	58.03±9.42#	23.15±10.01#	38.83±6.22#	0.73 ± 0.14#

Table 4: *Effect of the tested drugs on liver and kidney functions in alloxan-induced diabetic rats after 4 weeks. Values shown are means ± SEM; n = 5 rats per group. *: P < 0.05, significantly different from normal control group; #: P < 0.05, significantly different from diabetic control group.*

Myocardial biomarkers

Bromocriptine and cabergoline treated animals for 1 month in doses 10 mg/kg and 0.6 mg/kg resp. displayed significant elevation of LDH-1 level in the serum. By contrast, using combination of bromocriptine and cabergoline with sarpogrelate treatment significantly decreases the biomarkers level in the serum, compared with that of drugs used individually groups. The results were shown in the table

5. The qualitative test of troponin I reagent kit showed only positive results with groups of bromocriptine and cabergoline treated alone. ELISA results indicated that the expression levels of TNF-alpha 1 in the diabetic rats groups treated with bromocriptine and cabergoline alone were significantly higher than those in the diabetic control group. The groups of the rats of combination between bromocriptine and cabergoline with sarpogrelate had lower levels of TNF-alpha 1 expression than those in the groups treated with bromocriptine or cabergoline individually (Table 5).

Biochemicals		
Group	**LDH (IU/L)**	**TNFα1 (pg/mL)**
Normal control group	17.42 ± 0.69	06.12 ± 0.45
Diabetic control group	18.50 ± 1.53	08.32 ± 0.56
Diabetic group treated with Bromocriptine	40.50 ± 10.69#	36.56 ± 2.32#
Diabetic group treated with Cabergoline	36.86 ± 12.47#	31.20 ± 4.23#
Diabetic group treated with Domperidone	19.39 ± 19.39	07.34 ± 0.49
Diabetic group treated with Sarpogrelate	19.55 ± 2.20	09.74 ± 0.98
Diabetic group treated with Bromocriptine +sarpogrelate	27.75 ± 3.91a	14.84 ± 1.23a
Diabetic group treated with Cabergoline + sarpogrelate	34.57 ± 6.60b	08.59 ± 0.76b
Diabetic group treated with Cabergoline + domperidone	44.67 ± 6.10#	27.52 ± 4.23#

Table 5: *Effect of the tested drugs on Lactate dehydrogenase-1 and TNFα1 in alloxan-induced diabetic rats. Values shown are means ± SEM; n = 5 rats per group. #: P < 0.05, significantly different from diabetic control group. [a]: P < 0.05, significantly different from diabetic group treated with bromocriptine. [b]: P < 0.05, significantly different from diabetic group treated with cabergoline.*

Evaluation of myocardial injury

Myocardial infarct size can be an indicator of myocardial injury. Both of bromocriptine 4 mg/kg and cabergoline 0.6 mg/kg treated group hearts showed a significant increase of risk area infarct. By contrast, combination of bromocriptine or cabergoline with sarpogrelate (50 mg/kg) reduced the percentage of the myocardial infarct size (Figure 1A and 1B). The high noted adverse effect of bromocriptine and cabergoline on heart represented in myocardial injury and infarction was approved in the present study by using overdoses for long duration of treatment. It may relate to dopamine D_2 and serotonin 5-HT (2B) receptors agonistic properties which increase rate of the heat beating. It has been reported that bromocriptine and cabergoline (ergot derivative) have been associated with valvular heart disease, since the two drugs have both potent D_2 receptor and serotonin 5-HT(2B) receptor agonistic properties [12,13]. According to the current data, combination of bromocriptine and cabergoline with the new chemical agent sarpogrelate (selective 5HT2a/2b antagonists) decrease the adverse effects of these two drugs on heart. The protection effects of sarpogrelate on myocardial tissue were approved by its ability to decrease secretion of myocardial biomarkers like LDH-1, Troponin I and TNF alpha 1 during long term duration of treatment of bromocriptine and cabergoline in model of diabetic nephropathy rats. Sarpogrelate attenuates cardiac dysfunction, infarct size due to MI. These results also support the view that serotonin and 5-HT(2A) may contribute to the deleterious effects of ischemic injury in the heart. Regarding to the biochemical study, sarpogrelate drug can be consider as a safe drug on liver and kidney functions.

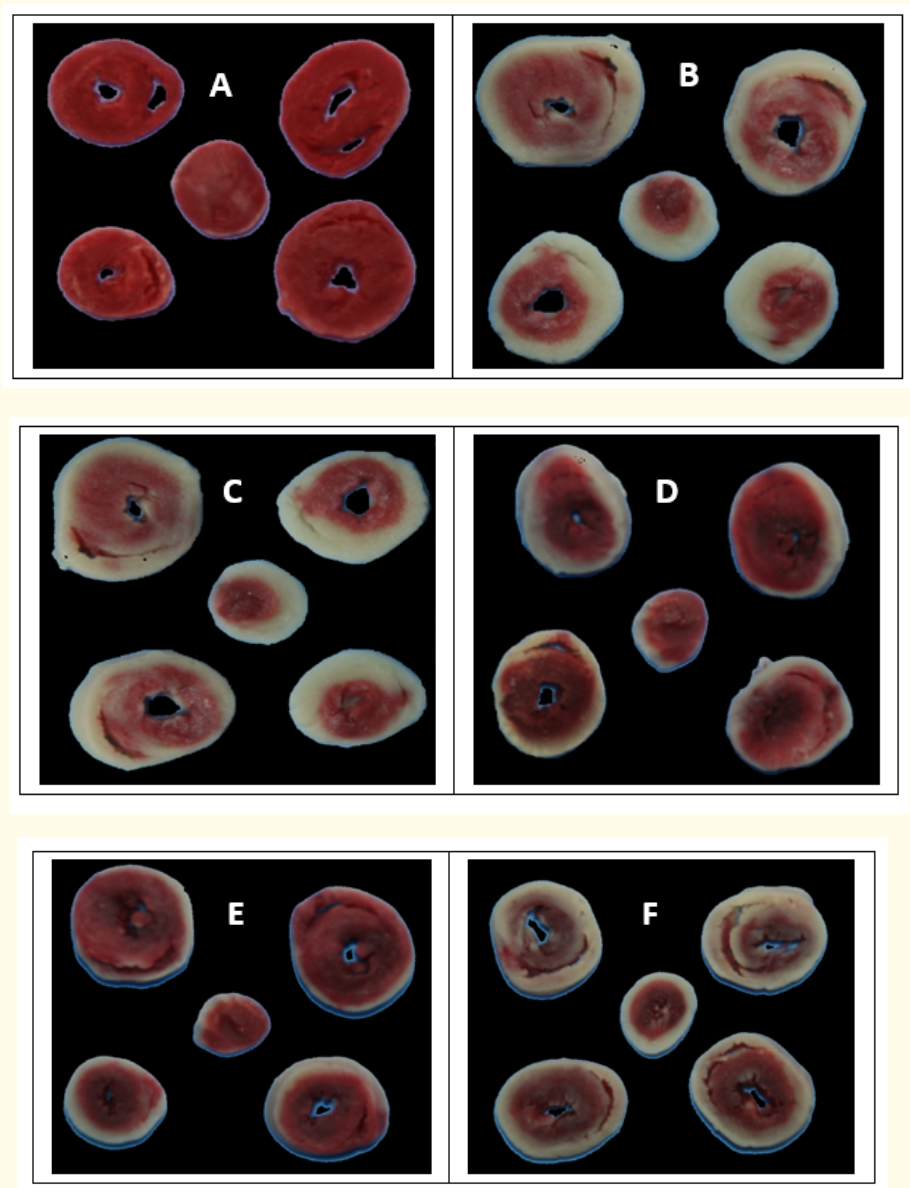

Figure 1A: *Effect of the tested drugs in alloxan-induced diabetic mice after 4 weeks of treatment. (A) Diabetic control rats, (B) Bromocriptine treated group, (C) Cabergoline treated group, (D) Bromocriptine+ Sarpogrelate treated group, (E) Cabergoline+ Sarpogrelate treated group, (F) Cabergoline+ domperidone treated group.*

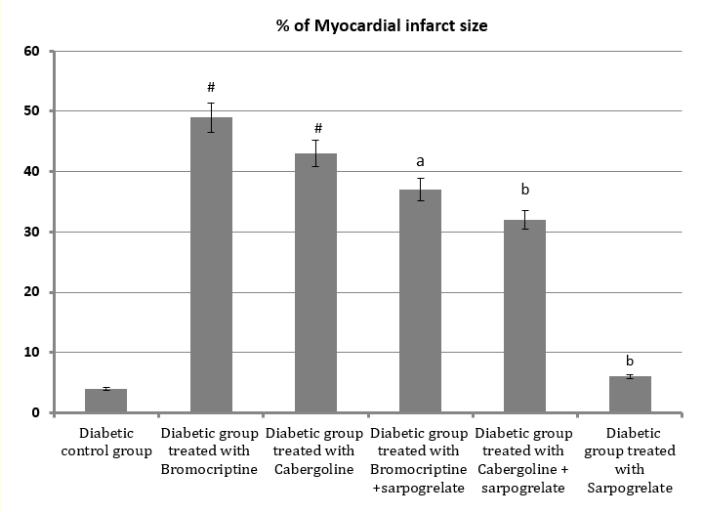

Figure 1B: *Effect of the tested drugs on percentage of myocardial infarct size in alloxan-induced diabetic rats. Values shown are means ± SEM; n = 5 rats per group. #: P < 0.05, significantly different from diabetic control group. a: P < 0.05, significantly different from diabetic group treated with bromocriptine. b: P < 0.05, significantly different from diabetic group treated with cabergoline.*

Evaluation of myocardial injury

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