Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/v4sc6764

RENOPROTECTIVE EFFECTS OF BROMOCRIPTINE WITH ANALYSIS OF RENAL MARKERS IN DIABETIC RATS: A MORPHOLOGICAL AND HISTOPATHOLOGICAL EVALUATION IN KIDNEY TISSUES

Sonia Khan[1*], Syed Shahzil Ali Shah[2], Asma Shabbir[3], Shahid Maqbool Korai[4], Naveen Aleem[5], Rubbiya Javed[6], Sajeela Furqanullah[7], Marvi[8]

[1*]Associate Professor, Department of Pharmacology, Al-Tibri Medical College, Karachi, Pakistan
[2] Final year MBBS Student, Ziauddin Medical College, Karachi, Pakistan
[3]Associate Professor, Department of Pathology, Jinnah Sindh Medical University, Karachi, Pakistan
[4]Assistant Professor, Department of Anatomy, Al-Tibri Medical College, Karachi, Pakistan
[5,6,7] Lecturer, Department of Anatomy, Al-Tibri Medical College, Karachi, Pakistan
[8]Assistant Professor, Department of Pharmacy, University of Baluchistan, Quetta, Pakistan.

*** Corresponding Author:** Sonia Khan.
*Email: drsonia.azeem2@gmail.com.

ABSTRACT

Background: Diabetes is one of the disorders that require life time management. Oral hypoglycemic are widely utilized by diabetic individuals to control sugar level. An abnormality in kidney function
markers is one of the major problems.

Objectives: Bromocriptine activates dopamine receptors in the kidney, which can help improve renal function, act as therapeutic agent for patients with diabetic nephropathy and used as part of a renal protective strategy to prevent or slow kidney disease progression.

Study Design: Experimental.

Place and Duration: Study was conducted at Karachi University, Department of Pharmacology, from November 2022 to April 2023.

Methods: An in vivo study was conducted to evaluate the effects of bromocriptine on diabetes and kidney damage. Blood samples from normal and diabetic male rats were analyzed for kidney markers i.e urea, creatinine, electrolytes and compared to a control diabetic group. The results showed that bromocriptine effectively reversed diabetic nephropathy in the diabetic-induced group, demonstrating its potential in managing diabetes and protecting the kidney.

Results: In this, bromocriptine was found to improve blood glucose level and significantly normalize renal markers compared to control diabetic group. Moreover, diabetic induced group caused substantial damage to renal architecture, which was notably reversed by bromocriptine. In conclusion, bromocriptine demonstrates potential in treating diabetes and protecting the kidney from diabetes-induced damage **Conclusion:** The studied concluded that bromocriptine have marked good antidiabetic activity and protecting the kidney from diabetic complications.

Key words: Bromocriptine, antidiabetic, diabetic nephropathy, urea, creatinine, electrolytes.

INTRODUCTION

Diabetes is a prevalent and insidious health threat in Pakistan, leading to numerous complications. Diabetes mellitus (DM) is a chronic disease, which rapidly increases due to lifestyle changes and some environmental factors. World Health Organization projected that diabetes will be the 7th leading cause of death in 2030.[1] Diabetes can be found in newborns and the incidence is higher in females than males.[1,2,3] . Bromocriptine has been shown to protect against renal fibrosis, a common complication of chronic kidney disease [4] Bromocriptine has been shown to reduce oxidative stress in the kidneys, which can contribute to renal damage [5]

Bromocriptine has anti-inflammatory properties, which can help reduce inflammation in the kidneys and slow the progression of kidney disease [6,7]. *Bakris* et al reported that bromocriptine has been shown to decrease proteinuria, a marker of kidney damage [8,9]. Cycloset (bromocriptine) is a sympatholytic D2-dopamine agonist that has been approved for the treatment of type 2 diabetes [10,11]. Millan MJ et al evaluate that the Cycloset (bromocriptine) is indicated for the management of type 2 diabetes [13,14,15]. Luo S et al suggested that bromocriptine has the potential to reverse metabolic disruptions associated with insulin resistance and obesity by reorganizing the hypothalamic circadian system and monoamine neuronal function.[16] Additionally, dopamine agonist treatment exerts its effects by reducing the hypothalamic signals that regulate hepatic glucose production, lipid synthesis, and insulin resistance[17,18].

Cycloset does not have a specific receptor that mediates its action on glucose and lipid metabolism. Rather, its effects are mediated via resetting of dopaminergic and sympathetic tone within the CNS [19]. This study explored the potential synergistic effects of cycloset (bromocriptine), a dopamine D2 receptor agonist that effectively reduced postprandial plasma glucose levels. This study investigates the nephroprotective activity in a rat model of bromocriptine induced diabetes, evaluating the efficacy of different doses of bromocriptine.

MATERIALS AND METHOD

Invivo Study

In this the study, male Wistar rats (200-250 g) were used, allocated into five groups of 10 animals each and maintained in a temperature- and humidity-controlled facility (n=50):
Group 1: Normal control group. (normal saline).
Group 2: Diabetic control group. (streptozotocin, 55 mg/kg).
Group 3: Diabetic treated group. (*cycloset,low dose* 1.8mg/kg)
Group 4: Diabetic treated group. (*cycloset,high dose* 4mg/kg)
Group 5: Diabetic treated group (Standard drug, 200mg/kg metformin)

After the end of 3 months, cardiac puncture has done to collect blood samples from the rats for various biochemical analyses.

Biochemistry Analysis: Blood samples of normal and diabetic male rat was collected (Allain et al,1974) and taken for analysis by centrifugation for 20min. centrifugation was done at 4°C, and stored at−20°C. urea,creatinine and electrolytes were analysed.

Preparation of kidney Tissue for Histological Examination Animal tissues were rinsed with saline and preserved in 10% neutral buffered formalin for histopathological analysis. After fixation, the tissues were embedded in paraffin wax using standard methods. Sections (5 μm) were prepared from the paraffin blocks and mounted on Poly-L-lysine-coated glass slides. The sections were then stained with H&E using standard protocols and examined by light microscopy to evaluate histological changes.

Place and Duration: Study was conducted at Karachi University, Department of Pharmacology, from November 2022 to April 2023.

Ethical Approval: The project has approved by ASRB (Advance study and research board) with (ASRB/No./06788/PHARM) on 17 October 2022.

Statistical Analysis: Statistical analysis was performed using SPSS 20.0 software (IBM SPSS, USA). A significance level of $p < 0.05$ was adopted for all two-tailed tests. Normally distributed continuous variables with homogeneous variance are presented as mean ± standard deviation (SD) and were compared using one-way analysis of variance (ANOVA).

RESULTS

Effect on Urea

The urea level of control non-diabetic rat was32.1 ± 1.91 mg/dl and of control diabetic was 30.6± 0.03 mg/dl, which after bromocriptine became26.6 ± 1.3 mg/dl (low dose) 24.3 ± 0.41 mg/dl (high dose). the urea level after standard drug treatment became 30.8± 0.35 mg/dl.

Effect on Creatinine

The creatinine level of control non-diabetic rat was0.445 ± 0.9 mg/dl and of control diabetic was 0.453± 1.03 mg/dl, which after bromocriptine became0.422 ± 0.3 mg/dl (low dose) 0.287 ± 1.41 mg/dl (high dose). the creatinine level after standard drug treatment became 0.5± 2.35 mg/dl.

Effect on Electrolytes

The sodium level of control non-diabetic rat was 144 ± 0.09 mEq/l and of control diabetic was 126 ± 0.58 mEq/l, which after bromocriptine became130 ± 2.3 mEq/l (low dose) 141 ± 0.41 mEq/l (high dose). the sodium level after standard drug treatment became 145± 0.25 mEq/l.

The potassium level of control non-diabetic rat was6.4 ± 1.81 mEq/l and of control diabetic was 6.0 ± 0.03 mEq/l, which after bromocriptine became6.5 ± 1.6 mEq/l (low dose)6.3 ± 1.61 mEq/l (high dose). the potassium level after standard drug treatment became 6.0± 0.35 mEq/l.

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The chloride and bicarbonate level of control non-diabetic rat was 107±1.9 mEq/l, 26.5± 1.91 mEq/l and of control diabetic was 129±1.7 mEq/l, 31 ± 0.03 mEq/l respectively, which after bromocriptine became 108 ±1.3 mEq/l, 26.1±2.47 mEq/l at low dose and 105 ± 0.4 mEq/l, 26.9± 1.8 mEq/l at high dose. The chloride and bicarbonate level after standard drug treatment became 106± 0.35 mEq/l and 29.8±1.5 mEq/l.

Table: Renal markers of control and treated groups of rats

RENAL MARKERS	Control non diabetic	Control diabetic	diabetic + bromocriptine (low dose)	diabetic + bromocriptine (high dose)	diabetic+ Standard treated
UREA	32.1 mg/dl	30.6mg/dl	26.6*^ mg/dl	24.3*^ mg/dl	30.8 mg/dl
CREATINIE	0.445.3 mg/dl	0.453mg/dl	0.422 mg/dl	0.287^ mg/dl	0.5 mg/dl
SODIUM	144 mEq/l	126 mEq/l	130*^ mEq/l	141^ mEq/l	145 mEq/l
POTASSIUM	6.4mEq/l	6.0 mEq/l	6.5 mEq/l	6.3 mEq/l	6.0 mEq/l
CHLORIDE	107 mEq/l	129 mEq/l	108 mEq/l	105 ^ mEq/l	106 mEq/l
BICARBONATE	26.5 mEq/l	31 mEq/l	26.1 mEq/l	26.9*^ mEq/l	29.8 mEq/l

Values are given in mean standard deviation is equal to significant at $p < 0.05$.
values shown with * are compared with control non diabetic group as significant.
values shown with ^ are compared with control diabetic group as significant.

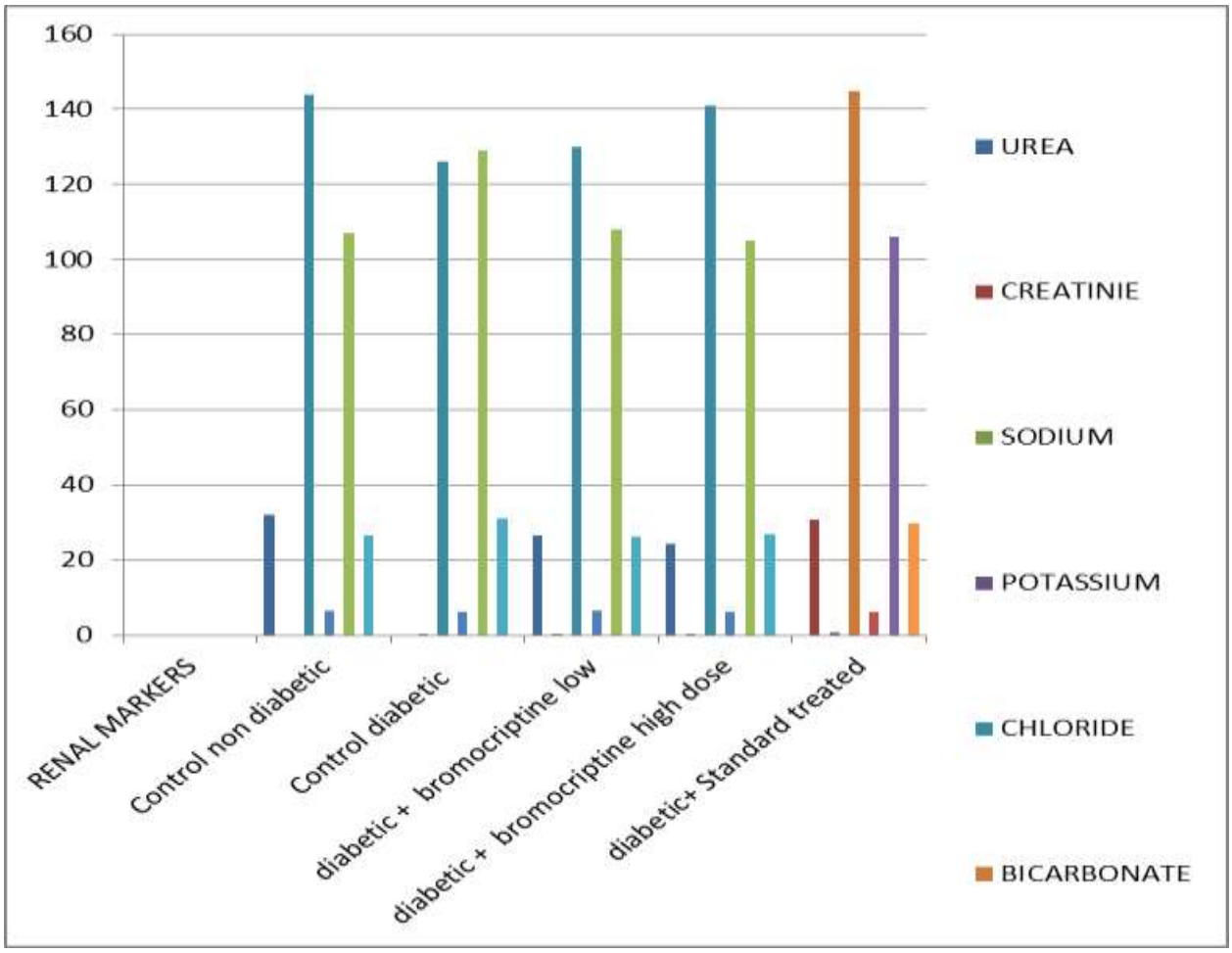

Figure 1: Graph showed the effect of bromocriptine and standard drug on RENAL MARKERS

Histopathological Results of Kidney

Control Non diabetic Group

Microscopic studies showed the normal spherical shape glomeruli and basement membrane. Renal tubules are lined by a single layer of epithelial cells and have a normal, uniform diameter as shown in Fig 2.

Control diabetic Group

Microscopic studies showed narrowed and atrophied renal tubules with a decreased diameter and mild to moderate inflammation and infiltration of lymphocytes as shown in Figure 3.

Diabetic treated Group (bromocriptine)

Microscopic studies showed that the kidney architecture was generally preserved with mild inflammation and infiltration of lymphocytes and intact renal tubules as shown in Fig 4.

Diabetic treated Group (standard group)

Microscopic studies showed the normal renal tubules lined by a single layer of epithelial cells with no evidence of inflammation or fibrosis and have a normal, uniform diameter as shown in Fig in Fig 5.

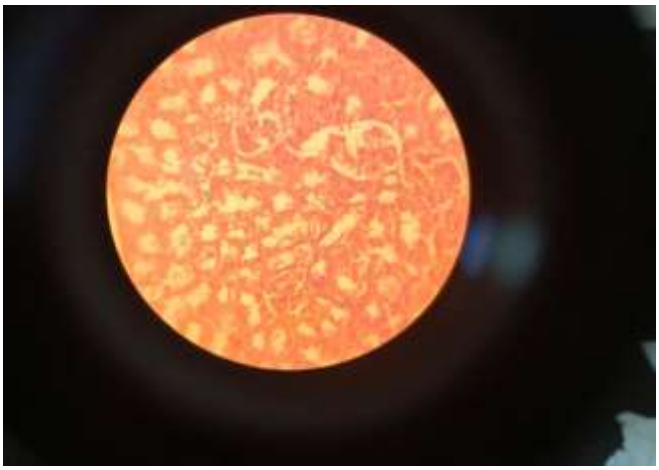

Fig. 2: 40X Photomicrograph of kidney showed normal spherical shape glomeruli and basement membrane. Renal tubules are lined by a single layer of epithelial cells. No significant change in control non-diabetic rat

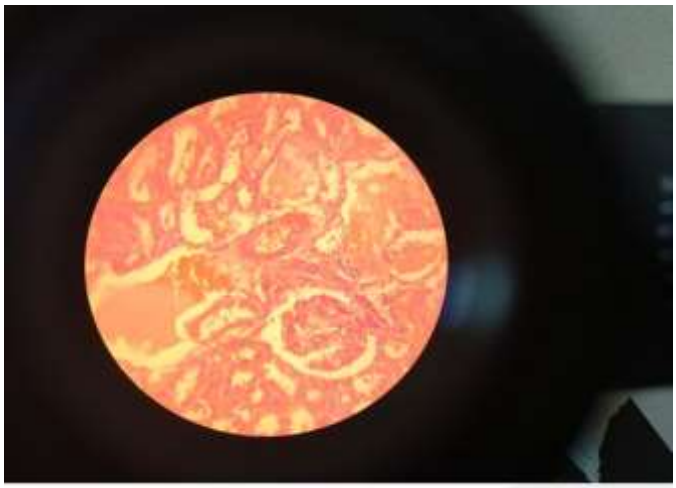

Fig. 3: 40X Photomicrograph of kidney showed narrowed and atrophied renal tubules with a decreased diameter and mild to moderate inflammation and infiltration of lymphocytes

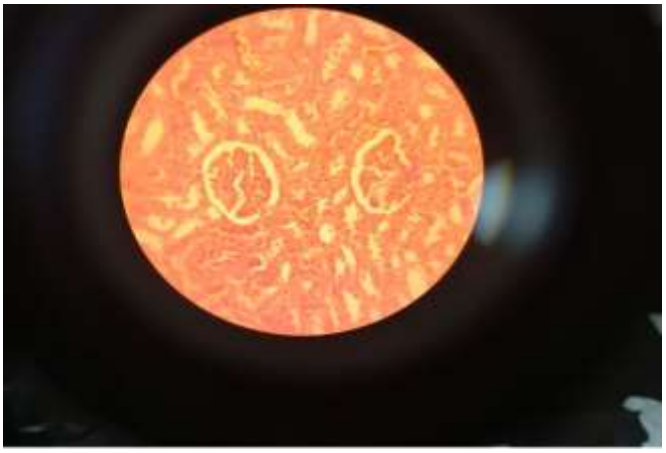

Fig. 4: 40X Photomicrograph of kidney of STZ treated group with bromocriptine showing kidney architecture was generally preserved with mild inflammation and infiltration of lymphocytes and intact renal tubules

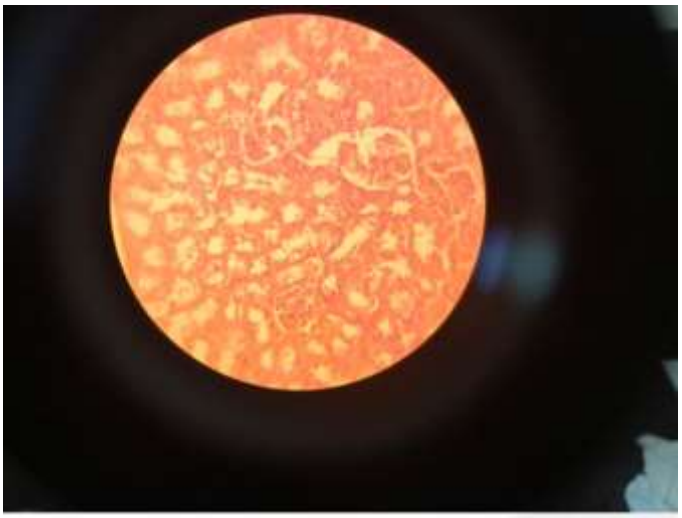

Fig. 5: 40X Photomicrograph of kidney with standard drug showing renal tubules lined by a single layer of epithelial cells with no evidence of inflammation or fibrosis

DISCUSSION

The incidence of diabetes mellitus is rising rapidly worldwide, frequently giving rise to severe metabolic disturbances and life-threatening complications [21]. Fletcher et al stated that Insulin resistance is a critical risk factor for the development of impaired glucose tolerance and type 2 diabetes. Individuals with insulin resistance often exhibit a cluster of risk factors, including hyperinsulinemia, atherogenic dyslipidemia and glucose intolerance, which are also commonly observed in people with type 2 diabetes. [22] The secondary outcomes of this study will examine the impact on cardiovascular disease and its associated risk factors, as well as changes in glycemic control, insulin dynamics, obesity, physical activity, nutrient intake, quality of life and the incidence of adverse events [23,24]. G M Reaven supported the view that the concurrent presence of insulin resistance and hyperinsulinemia sets the stage for the emergence of a constellation of metabolic abnormalities, characterized by impaired glucose tolerance, elevated plasma triglycerides, reduced high-density lipoprotein cholesterol, and hypertension [25].

Carey et al. discusses the role of dopamine receptors in the kidney and their potential therapeutic target for treating renal diseases. The authors highlight the importance of dopamine D2 receptors in regulating renal function and blood pressure. Bromocriptine, a dopamine D2 receptor agonist, is mentioned as a potential therapeutic agent for treating hypertension and renal disease. [26] Zhang et al. investigated the effects of bromocriptine on renal function in patients with chronic kidney disease. The results show that bromocriptine treatment leads to significant reduction in serum urea and creatinine levels and improvement in renal function. Cincotta et al demonstrated that Cycloset has a positive impact on metabolic parameters, reducing insulin resistance and glucose intolerance and improving hyperglycemia in obese individuals with type 2 diabetes [26]. Li et al. evaluated the effects of bromocriptine on serum creatinine and urea levels in patients with chronic kidney disease. The results showed that bromocriptine treatment leads to significant reduction in serum creatinine and urea levels. [27].

According to the findings of the study, administration of bromocriptine for 12 weeks significantly normalize renal marker as shown in table 1. The study's findings revealed a significant decrease in urea level when treated with bromocriptine with low dose (26.6 ± 1.3U/) and (24.3 ± 0.41U/L) high dose showing substantial reductions as compared to diabetic treated group. When bromocriptine was given to treated group sodium and chloride level maintained and p-value (0.0001) gives significant difference with diabetic treated group. When bromocriptine was given in diabetic group, creatinine was not much altered near to standard treated group So it is analyzed that bromocriptine has positive effect on renal marker and has nephroprotective effect in diabetic treated group. Histopathological examination of kidney tissue revealed that and atrophied renal tubules with a

decreased diameter and mild to moderate inflammation and infiltration of lymphocytes positive control group (Fig. 3) Notably, these changes were significantly reversed in the bromocriptine treated groups. (Fig.4,5)

CONCLUSION

This study demonstrates the renoprotective effects ofbromocriptine, offering a novel, natural therapeutic approach for managing diabetic nephropathy.

Conflict of Interest

No conflict of interest associated with this work.

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