TO STUDY THE EFFECTS OF COMBINATION OF ZINC AND DAPAGLIFLOZIN IN TYPE 2 DIABETES IN ALBINO RATS

Muhammad Sair[1], Syeda Afroz[2], Shireen Nazir[3], Saba Jawaid[4], Wardha Saad [5], Rushda Fatima[6], AzmatAra[7].

[1]Associate Professor, Department of Pharmacology, M.Islam Medical & Dental College Gujranwala, Pakistan.

[2]Associate Professor, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

[3]Assistant Professor, Department of Pharmacology, Altamash Institute of Dental Medicine, Clifton, Karachi, Pakistan

[4]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

[5]Research scholar, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

[6]Research scholar, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

[7]Research scholar, Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Science, University of Karachi, Karachi, Pakistan

ABSTRACT

Aim:

This study examined the combined administration of zinc and Dapagliflozin in albino rats with induced insulin-dependent diabetes. SGLT2 inhibitors, a novel diabetes treatment, were investigated for their potential to reduce glycosylated hemoglobin by inhibiting sodium and glucose reabsorption. The research aimed to understand the synergistic impact of zinc, crucial for insulin function, along with Dapagliflozin in managing diabetes in the rat model.

Methodology: The study, conducted at the Department of Pharmacology, M. Islam Medical College, Gujranwala, spanned three months and involved 50 albino rats categorized into five groups. Insulin-dependent diabetes mellitus was induced in all rats, excluding those in group 1, using streptozotocin. Blood samples were collected at the initiation of the study and repeated at months 1, 2, and 3. HbA1c levels were assessed at the study's commencement and at the end of the third month.

Results:

- **Group 1:** All measurements for Fasting Blood Sugar (FBS), Random Blood Sugar (RBS), and HbA1c remained within the normal range.
- **Group 2:** Induced with streptozotocin and Nicotinamide, displayed elevated RBS and FBS levels. By day 90, HbA1c indicated poor glycemic control.
- **Group 3:** Treated with Dapagliflozin, exhibited positive responses, with FBS, RBS, and HbA1c remaining within normal limits.

- **Group 4:** Administered zinc, showed partial effects with a slight decrease in FBS and RBS, although they remained high. HbA1c indicated poor glycemic control but was slightly better than in Group 2.
- **Group 5:** The combination of Dapagliflozin and zinc yielded significantly better responses compared to Group 4.

Conclusion: The combined administration of Dapagliflozin and zinc demonstrated improved outcomes in glycemic control compared to individual treatments. The positive responses observed in Group 5 suggest a potential synergistic effect of Dapagliflozin and zinc in managing diabetes in albino rats. This highlights the importance of exploring combination therapies for enhanced therapeutic outcomes in diabetes management.

Keywords: Dapagliflozin, Albino rats, HbA1c, Diabetes Mellitus, Zinc

INTRODUCTION:

Sodium-glucose cotransporter-2 inhibitors (SGLT2) function by inhibiting the reuptake of sodium and glucose in the proximal tubule, resulting in heightened glucose excretion in urine. These inhibitors are recognized for reducing glycosylated hemoglobin (HbA1c) by approximately 0.5%–0.7% and contributing to a weight loss of 2–3 kg (1). SGLT1, crucial for intestinal glucose absorption, is the primary mechanism for lumen-to-cell glucose uptake (2). Currently, four SGLT2 inhibitors—Canagliflozin, Dapagliflozin, Empagliflozin, and Ertugliflozin—have been approved since 2013, and combination therapies, including metformin or a dipeptidyl peptidase-4 (DPP-4) inhibitor, are available for type 2 diabetes patients (3).

This relatively new class of diabetes drugs, SGLT2 inhibitors, exhibits promise in slowing diabetic kidney disease progression and improving cardiovascular outcomes by effectively controlling glycemia through increased urinary glucose excretion and reduced blood glucose levels (4). However, their usage has been linked to a mild-to-moderate increase in urinary tract infections, primarily with Candida species, representing the most common adverse effect associated with SGLT2 inhibition (5).

Considering the global prevalence and impact of diabetes, there is a crucial need for innovative therapeutic strategies for type 2 diabetes patients (6). SGLT2 inhibitors are considered potential pharmacological treatments as second-line therapy following metformin failure or intolerance, with Empagliflozin or Canagliflozin preferred as second-line therapies for patients unable to achieve glycaemic control on monotherapy.

In diabetic cohorts, zinc content has been reported to decrease by 75%, and supplementation has demonstrated improvement in diabetic symptoms (9). Zinc deficiency is observed in both human and animal diabetics, playing a vital role in the immune system and preventing free radical formation (11). The high global mortality and morbidity associated with diabetes, with an 8.5% prevalence, is anticipated to affect 300 million people by 2030, with a 10% prevalence in Pakistan. The significance of zinc in enhancing glucose tolerance, reducing blood glucose levels, and influencing the expression of diabetes-related genes underscores its importance (12).

As a crucial trace element, zinc significantly contributes to oxidative stress by acting as an antioxidant and is an essential component of superoxide dismutase and various metallothioneins, enhancing the antioxidant system in the human body (13). Moreover, zinc is indispensable for the conversion of proinsulin to insulin and plays a critical role in maintaining normal insulin function, production, and storage (16).

Streptozotocin (STZ) is widely employed to induce insulin-dependent diabetes mellitus in experimental animals due to its toxic effects on islet beta cells (17). The diabetogenic action of STZ results in irreversible damage to pancreatic beta cells, leading to degranulation and loss of the capacity to secrete insulin (18). An effective dose of 45 mg/kg of STZ induces hyperglycemia, weakness, and reduced body weight due to its injurious effects on DNA, causing hyperglycemia and necrotic lesions (19). The commonly used diabetes model involves administering a single high dose of STZ to animals, resulting in the destruction of pancreatic beta cells, hyperglycemia, and deficient insulin production (21). Although experimental models closely resembling human type 2 diabetes (DM2) are challenging, studies have shown that mice fed a high-fat (HF) diet develop symptoms related to DM2, including insulin resistance and hyperinsulinemia, albeit not hyperglycemia (22).

MATERIAL AND METHODS

The study was conducted under the supervision of Dr. Afroz at the University of Karachi in collaboration with the Department of Pharmacology at Shahida Islam Medical College, Lodhran.

Experimental Design:

Fifty albino rats were obtained from the animal facility of Shahida Islam Medical College, divided into five groups, each comprising 10 rats. Except for Group 1, all rats were induced with diabetes using streptozotocin at a dose of 65 mg/kg as a 1 ml freshly prepared solution in 0.1 m citrate buffer with a pH of 4.5. To prevent drug-induced hypoglycemic mortality, the animals received 5% glucose water for 48 hours post-streptozotocin administration.

Data Collection:

Blood samples were systematically collected from each rat at the initiation of the study and repeated at 1, 2, and 3 months. HbA1c assessments were conducted at the study's commencement and the conclusion of the 3-month period.

Materials Used:

The materials included Dapagliflozin tablets (5mg, 10mg), Zinc oxide tablets (100mg), and Streptozotocin.

Parameters Measured:

Parameters measured encompassed Serum Fasting Blood Sugar (FBS), Serum Random Blood Sugar (RBS), and Hemoglobin A1C (HbA1c).

Inclusion and Exclusion Criteria:

Inclusion criteria involved healthy rats weighing between 150-200g, while exclusion criteria comprised rats weighing less than 150g.

Data Collection Procedures:

Data collection involved the systematic collection and organization of all data onto tables for subsequent analysis.

RESULTS:

Table 1: Grouping of Animals

Group No	Groups
1	Control group
2	STREP+NICO GROUP
3	Dapagliflozin group
4	Zinc group
5	Zinc + Dapagliflozin group

1. Control Group (Group 1):
    - All readings for Fasting Blood Sugar (FBS), Random Blood Sugar (RBS), and Hemoglobin A1c (HBA1c) in Group 1 remained within the normal range.
2. STREP+NICO Group (Group 2):
    - Introduction of streptozotocin and Nicotinamide to induce type 2 diabetes resulted in increased levels of RBS and FBS. By day 90, HBA1c indicated poor glycemic control.
3. Dapagliflozin Group (Group 3):
    - Dapagliflozin demonstrated positive responses in Group 3, with all parameters (FBS, RBS, and HBA1c) remaining within normal limits.
4. Zinc Group (Group 4):
    - Zinc administration showed partial effects. Although FBS and RBS saw a slight decrease, they remained in a high range. HBA1c indicated poor glycemic control but was slightly better than in Group 2.

5. Zinc + Dapagliflozin Group (Group 5):

    - The combination of Dapagliflozin and zinc in Group 5 yielded positive results. A comparison with Group 4 revealed that the combination in Group 5 exhibited significantly better responses.

In this study, five distinct groups of animals were carefully examined to assess the impact of different treatments on diabetes. The Control Group (Group 1) served as the baseline, demonstrating normal range readings and validating the overall health of the rats, ensuring the accuracy of the experimental conditions. In contrast, the STREP+NICO Group (Group 2) exhibited elevated levels of Random Blood Sugar (RBS) and Fasting Blood Sugar (FBS), coupled with poor glycemic control indicated by Hemoglobin A1c (HBA1c), confirming the successful induction of type 2 diabetes in this experimental cohort.

The Dapagliflozin Group (Group 3) showcased positive responses, with all measured parameters—FBS, RBS, and HBA1c—remaining within normal limits. This underscores the effectiveness of Dapagliflozin in maintaining glycemic control.

The Zinc Group (Group 4) demonstrated partial effects, with a modest decrease observed in FBS and RBS; however, these levels remained outside the normal range. Although there was an improvement in HBA1c compared to the induced diabetes group (Group 2), it suggested a potential but limited impact of zinc alone on glycemic control.

Remarkably, the Zinc + Dapagliflozin Group (Group 5) revealed superior results compared to the Zinc Group (Group 4). The combination of Dapagliflozin and zinc exhibited significantly better responses in all measured parameters, suggesting a synergistic effect in addressing diabetes. This finding implies that the combined approach may offer a more effective strategy for managing diabetes compared to individual treatments with Dapagliflozin or zinc alone.

Table 2: Fasting Blood Sugar (FBS) Measurements

Groups	FBS DAY 1	FBS DAY 30	FBS DAY 60	FBS DAY 90
G1	120	124	115	128
G2	198	200	250	220
G3	100	98	110	106
G4	189	180	200	200
G5	90	92	110	90

This table presents the Fasting Blood Sugar (FBS) measurements for each group at different time points (DAY 1, DAY 30, DAY 60, and DAY 90).

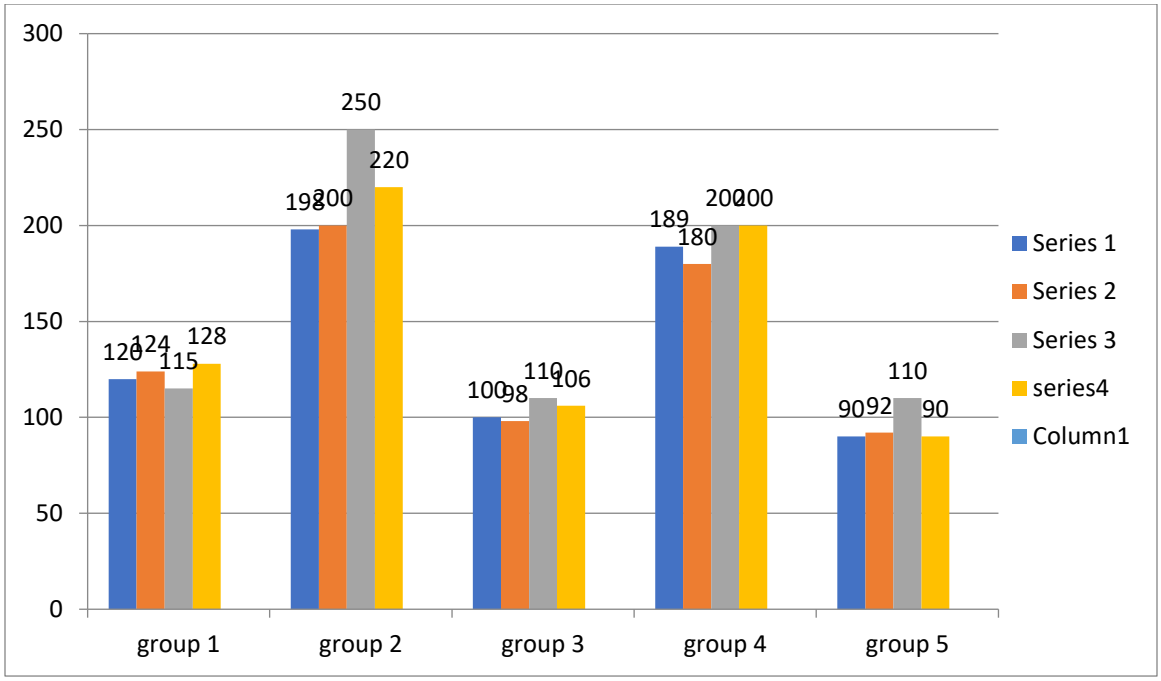

FIGURE 1: Fasting Blood Sugar (FBS) Measurements

Table 2: Random Blood Sugar (RBS) Measurements

Grouping	RBS DAY 1	RBS DAY 30	RBS DAY 60	FBS DAY 90
Group 1	190	192	196	186
Group 2	300	250	320	350
Group 3	150	155	140	152
Group 4	280	235	300	320
Group 5	140	130	142	146

This table presents the Random Blood Sugar (RBS) measurements for each group at different time points (DAY 1, DAY 30, DAY 60, and DAY 90).

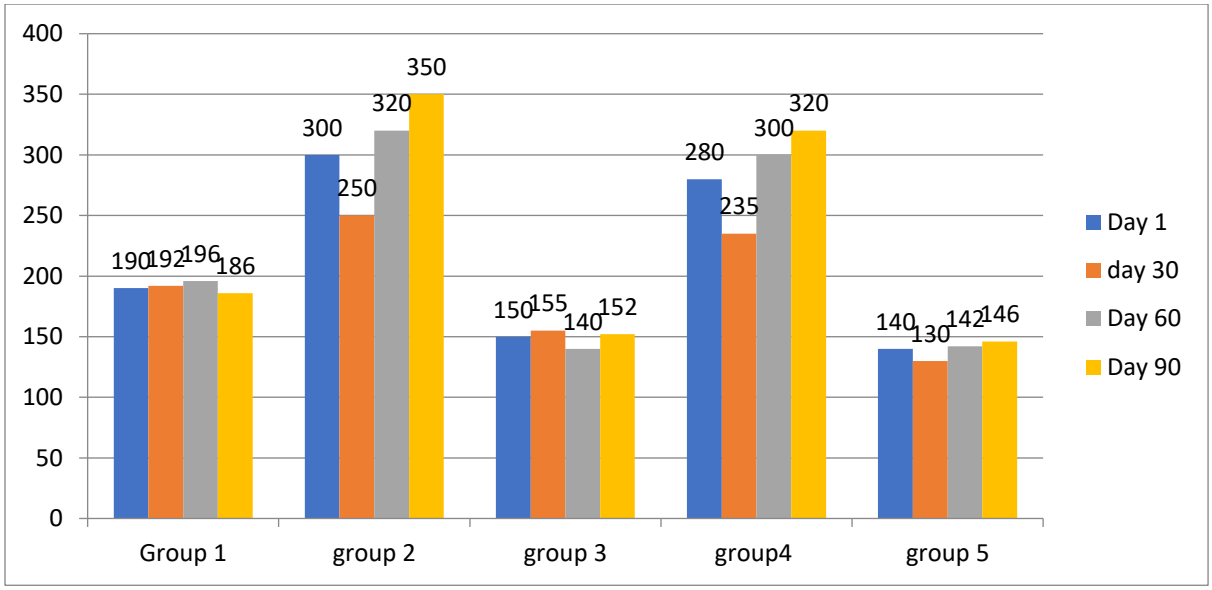

Figure 2: Random Blood Sugar (RBS) Measurements on Bar Chart

Table 3: Hemoglobin A1c (HBA1c) Measurements

Grouping	HBA1C DAY 1	HBA1C DAY 90
Group 1	3.2	3.0
Group 2	3.1	6.2
Group 3	3.2	4.2
Group 4	3.2	5.5
Group 5	3.2	3.4

This table presents the Hemoglobin A1c (HBA1c) measurements for each group at different time points (DAY 1 and DAY 90).

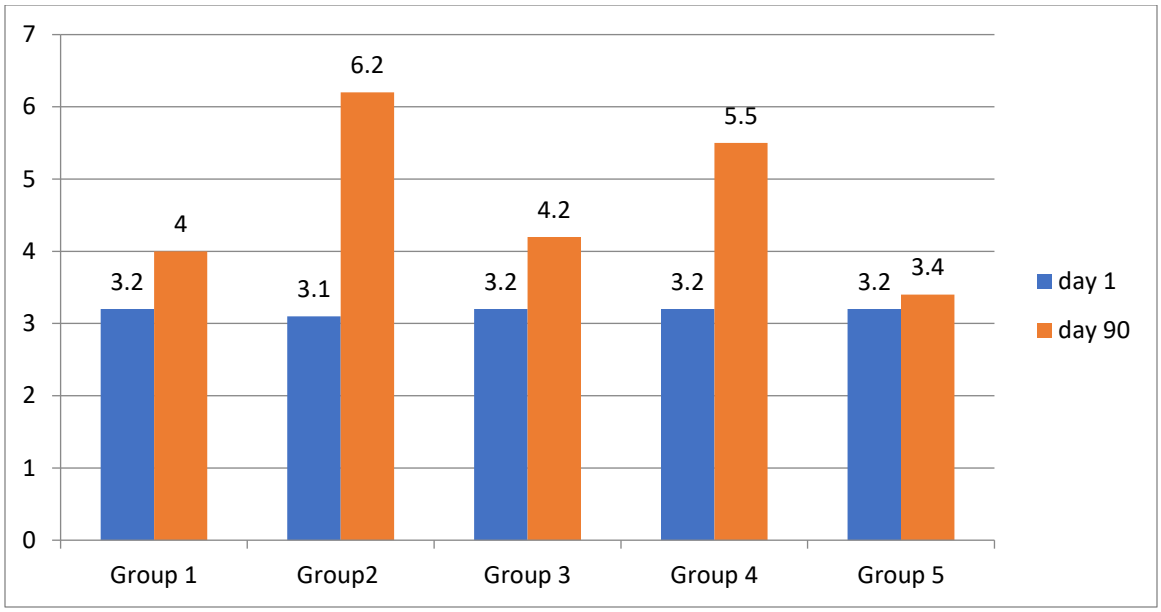

FIGURE 3: Hemoglobin A1c (HBA1c) Measurements on the Bar Chart

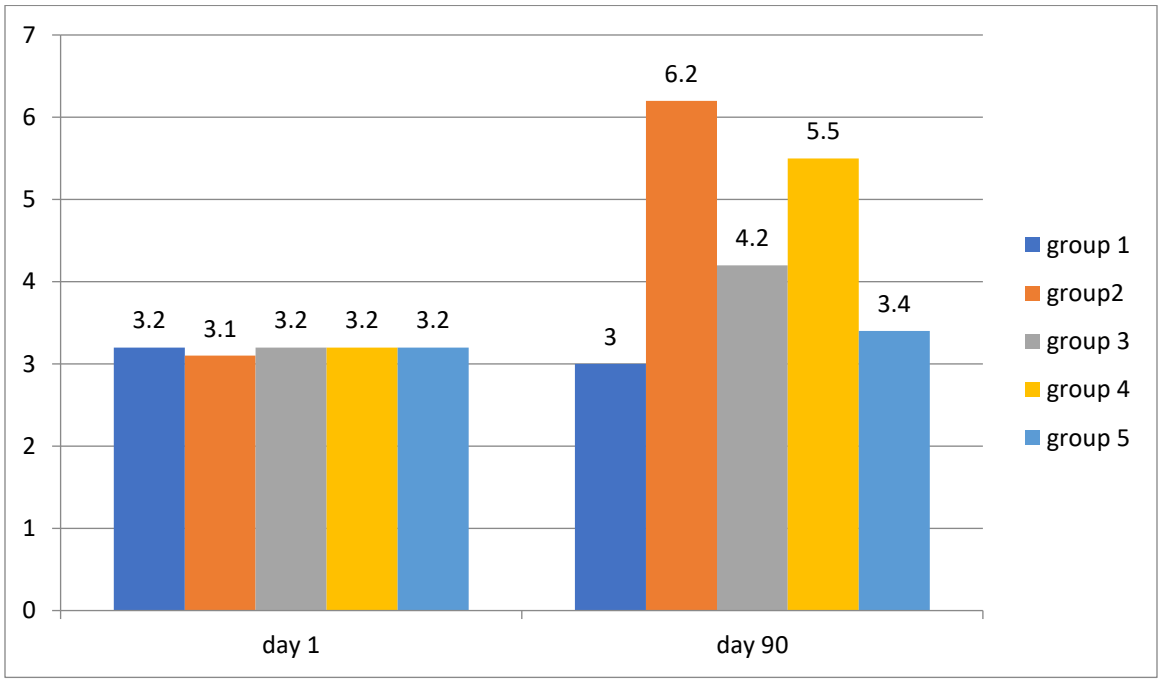

FIGURE 4: Comparison of HBA1c level in all five groups at day 1 and day 90

DISCUSSION

Our study observed that the combination of zinc demonstrated a slightly better response in Group 4 compared to Group 2, indicating improved glycemic control. This finding aligns with a study conducted by Farooq M et al. (18). Additionally, our results are consistent with another study by Al Maroof, where significant improvement in glycemic control was observed after three months of zinc administration (19).

The beneficial effects of zinc on glycemic control are further supported by existing literature, revealing a decrease in peripheral insulin resistance following zinc supplementation (20). Farooq M's study also reported significantly low levels of zinc in diabetic subjects (21).

Furthermore, our findings align with a study conducted by Susmita Barman, which concluded that zinc supplementation partially ameliorates the severity of oxidative stress induced by streptozotocin. This collective evidence underscores the potential positive impact of zinc in managing glycemic control and mitigating oxidative stress in diabetes.

CONCLUSION

In a noteworthy study, the simultaneous application of Dapagliflozin and zinc showcased superior results in regulating glycemic levels when contrasted with the effects of each treatment alone. Group 5's positive responses strongly indicate a promising synergistic influence between Dapagliflozin and zinc, particularly in the context of diabetes management for albino rats. This discovery emphasizes the critical significance of delving into combination therapies, shedding light on their potential to significantly improve therapeutic outcomes in the intricate landscape of diabetes management.

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