Research Article

# An Experimental Study to Evaluate the Effect of Antihyperglycemic Drugs on Serum Leptin and Adiponectin Levels in Streptozotocin-Induced Diabetes in Wistar Rats

**Rishabh Pandey[1*], Shweta Dwivedi[2], Mohd Shakir Hussain[3], Ashish Kumar Dixit[4], Amod Kumar Sachan[5], Satyendra Kumar Singh[6], Luv Arya[7]**

[1]Senior Resident, Department of Pharmacology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.
[2]Senior Resident, Department of Pharmacology, Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, India.
[3]Senior Resident, Department of Pharmacology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, 206130 Uttar Pradesh, India.
[4]Senior Resident, Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India.
[5]Professor & Head, Department of Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India.
[6]Additional Professor, Centre for Advance Research, King George's Medical University, Lucknow, Uttar Pradesh, India.
[7]Senior Resident, Department of Pharmacology, Uma Nath Singh Autonomous State Medical College, Jaunpur, India.
***Corresponding author's E-mail:** rishabsmu@gmail.com

**Received:** 25-05-2024; **Revised:** 28-08-2024; **Accepted:** 06-09-2024; **Published on:** 15-09-2024.

**ABSTRACT**

Diabetes mellitus is a widespread and dangerous global health issue that has arisen because of quick cultural, economic, and social changes, an aging population, and uncontrolled urbanization. The contemporary strategy involves assessing the impact of SGLT-2 inhibitors and their combination with Biguanide (Metformin) on blood glucose, body weight, and serum leptin and adiponectin levels in rats with diabetes induced by streptozotocin. To evaluate the effect of Dapagliflozin, Empagliflozin, Metformin, and their two-drug (SGLT-2 inhibitor + Biguanide) combination therapy on modulation of serum Leptin and Adiponectin levels in T2DM rat model. The current experimental investigation was carried out at the Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow. Adult male Wistar Rats were randomly divided into 7 groups, each group containing n=6 rats, and assessed for 9 weeks. Parameters like serum leptin and adiponectin, blood glucose, and body weight were assessed in response to Dapagliflozin, Empagliflozin, Metformin administered as monotherapy, and SGLT-2 inhibitors plus metformin administered as combination therapy in streptozotocin-induced diabetic rats. The mean weight, blood glucose, serum Leptin, and Adiponectin were assessed at the end of the 5$^{th}$ week (Baseline levels) and 9$^{th}$ week (Final reading). At week 9, the reduction in mean weight, mean blood glucose, mean serum leptin, and increase in mean serum adiponectin, was observed in all the drug-treated groups, but maximum and statistically significant reduction was observed in combination therapy (SGLT-2 inhibitors + Biguanide) as compared to their baseline levels of 5$^{th}$ week and as Compared to Group-II (Diabetic control) at 9$^{th}$ week. Based on current results the study concludes that SGLT-2 inhibitors and a combination of SGLT-2 inhibitors with metformin proved to be beneficial in strategies for treating Type 2 Diabetes patients associated with obesity.

**Keywords:** Adiponectin, Leptin, Streptozotocin, Diabetic model, SGLT-2 inhibitors, Metformin.

## INTRODUCTION

Diabetes mellitus is a persistent metabolic condition characterized by numerous aetiologies defined by persistent hyperglycaemia and poor regulation of blood glucose levels. This could be attributed to insufficient insulin secretion, resistance to peripheral action, or a combination of both[1]. Insulin resistance (IR) significantly contributes to its progression as well as to the emergence of other metabolic ailments like obesity, hypertension, elevated blood cholesterol, and metabolic syndrome[2]. From a biological perspective, IR is brought on by a high level of free fatty acids (FFAs), which deposit as triglycerides (TGs) in non-fat tissues such as skeletal muscles, the liver, heart, and pancreas. Molecularly, IR is caused by an imbalance between the cell membrane's receptors and extra nutrients or inflammatory cytokines[3,4].

Diabetes is predicted to affect 537 million people worldwide in 2021, 643 million by 2030, and 783 million by 2045. Additionally, it was anticipated that 541 million individuals would have impaired glucose tolerance by 2021[3]. In the global diabetes pandemic, India ranks second to China with a diabetic population of 77 million[4]. As of February 2022, there were 774,194,700 adult cases of diabetes in India, with an adult prevalence of 8.3%[5].

Insulin resistance is associated with visceral obesity, which is brought on by fat build-up in the omental and mesenteric areas; subcutaneous abdominal fat appears to be less associated with insulin insensitivity. The phrase "metabolically obese" refers to a large prevalence of Type 2 Diabetes Cases who are not visibly obese but have elevated visceral fat[5.6].

Leptin is a hormone mostly produced by adipocytes, with minor contributions from the brain, skeletal muscle, gut, placenta, and mammary epithelium[7]. It suppresses appetite by acting on the hypothalamus that's why leptin is known as an "anorexigenic" hormone. Nonetheless, obesity is distinguished by elevated leptin levels resulting from the emergence of leptin resistance, and the levels of circulating leptin are directly correlated with the overall fat content within the body[8,10]. Elevated leptin concentrations are associated with the initiation of insulin resistance, heightened cardiovascular risk, elevated incidence of microvascular complications, cardiac autonomic dysfunction, and carotid atherosclerosis in individuals with type 2 diabetes mellitus (T2DM)[21]. In obesity, there is a

contradictory rise in the serum leptin level, likely attributed to the expansion of fat mass, given that adipocytes produce and release leptin. Paradoxically, this heightened leptin level could potentially trigger leptin resistance, subsequently diminishing glucose utilization capacity and disrupting glucose metabolism.

When increased adipose leptin synthesis was seen in most of the obese people without an appropriate leptin-mediated end-organ response, the term "leptin resistance" was coined. Studies on obese animals suggested that leptin resistance is linked to reduced leptin-mediated JAK-STAT signalling, induction of suppressor of cytokine signaling-3 (SOCS-3), and impaired leptin transport across the blood-brain barrier (BBB). Leptin sensitivity is reduced in the brain, which causes an abnormal build-up of triglycerides in adipose tissue, muscle, liver, and pancreas, impairing insulin secretion & sensitivity[7].

Unlike leptin, adiponectin is an anti-inflammatory cytokine generated by adipocytes. It enhances insulin sensitivity and hinders the inflammatory process. Within the liver, it diminishes the expression of gluconeogenic enzymes and the rate of glucose synthesis, while in muscle, it promotes glucose transport and increases fatty acid oxidation, in part by activating AMP kinase[8]. Numerous disease conditions, like obesity, diabetes, and atherosclerosis, have been linked to reduced adiponectin concentrations[12].

However, the primary approach for managing individuals with T2DM involves lifestyle interventions, encompassing weight loss, dietary adjustments, and enhanced physical activity. When pharmacological intervention becomes necessary, metformin stands as the first-line treatment. Nevertheless, certain patients encounter intolerance to metformin owing to its side effects like diarrhoea and renal impairment, SGLT-2 inhibitors are recommended in these patients[13].

The newer drugs like SGLT-2 inhibitors are due to their unique mechanism of action, which causes glucosuria and controls fasting as well as post-prandial blood glucose independent of insulin action[14]. Weight loss may also be aided by glucose-based calorie loss in the urine. Additionally, osmotic diuresis brought on by glycosuria may be beneficial for patients with hypertension and fluid overload. They can also be taken at any point during T2DM, even after the endogenous insulin reserve has greatly diminished[7,9,15]. Metformin's main effect is on the liver, where it lowers hepatic glucose output, activates cyclic AMP kinase (cAMPK), the cell's fuel sensor, and hinders mitochondrial glycerol-3 phosphate dehydrogenase, thereby decreasing hepatic gluconeogenesis. It affects decreased hunger, weight loss, lowered blood pressure, and an improved lipid profile.

It has been noted that adipokines, like leptin, are thought to have pro-inflammatory effects on the myocardium, while others such as adiponectin exhibit anti-inflammatory and cardioprotective properties. SGLT-2 inhibitors have been postulated as a strategy for resetting the equilibrium between these pro- and anti-inflammatory adipokines[16]. Metformin acts as a leptin sensitizer not only in the brain[17], but also in the liver, and these findings may offer further justification for metformin's potential medicinal applications in illnesses linked to leptin resistance[18].

There are few studies on Metformin and SGLT-2 inhibitors on modulating the serum levels of leptin (pro-inflammatory cytokine), adiponectin (anti-inflammatory cytokine) and their combination. Hence, the current study is structured to examine the impact of SGLT-2 inhibitors and their combination with Biguanides (Metformin) on parameters such as blood glucose, body weight, serum leptin, and adiponectin levels in diabetic rats induced by streptozotocin.

## MATERIALS AND METHODS

This investigation took place at the Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow, to assess the impact of Dapagliflozin, Empagliflozin, Metformin and their two-drug (SGLT-2 inhibitor + Biguanide) combination therapy on blood glucose, Body Weight, Serum Leptin & Adiponectin levels in streptozotocin-induced diabetic rats, after receiving approval from the Institutional Animal Ethics Committee (IAEC) vide ethical clearance **Project no.151/IAEC/2021 dated 20/10/2021**.

### Animals

The experiments were conducted on adult Male Wistar rats weighing 160-200 grams. Forty-two rats were procured from CSIR-IITR, Gehru Campus, Lucknow, India. The animals were accommodated within the Institutional animal facility, adhering to standard housing conditions, including a room temperature ranging from 24-27°C, a humidity level of 60-65%, and a 12-hour cycle of light and darkness. The food in the form of dry pellets was given in two forms – normal pellet diet (NPD) and high-fat diet (HFD - 58% fat, 27.5% carbohydrate, and 14.5% protein, as a percentage of total kcal[19] acquired from Bharat Science Solution Company, Unnao, Uttar Pradesh. Water was available ad libitum. A one-week acclimatization period was granted to all animals before the commencement of the experiment.

**Drugs and chemicals** Dapagliflozin (DAPA) was obtained from INTAS Pharmaceuticals (India). Metformin (MET) was purchased from USV Private Limited (India) and Empagliflozin (EMPA) was purchased from Boehringer Ingelheim Private Limited (India). STZ was procured from Sigma-Aldrich. All additional chemical substances were of superior analytical quality.

### Induction of Type 2 Diabetes Mellitus

Animals were supplied with a high-fat diet (HFD) for 4 weeks except Group I (Normal control). Following four weeks of a high-fat diet, a fasting period was observed for all rats from 7 am to 3 pm. Subsequently, a sole intraperitoneal injection of Streptozotocin (STZ) was

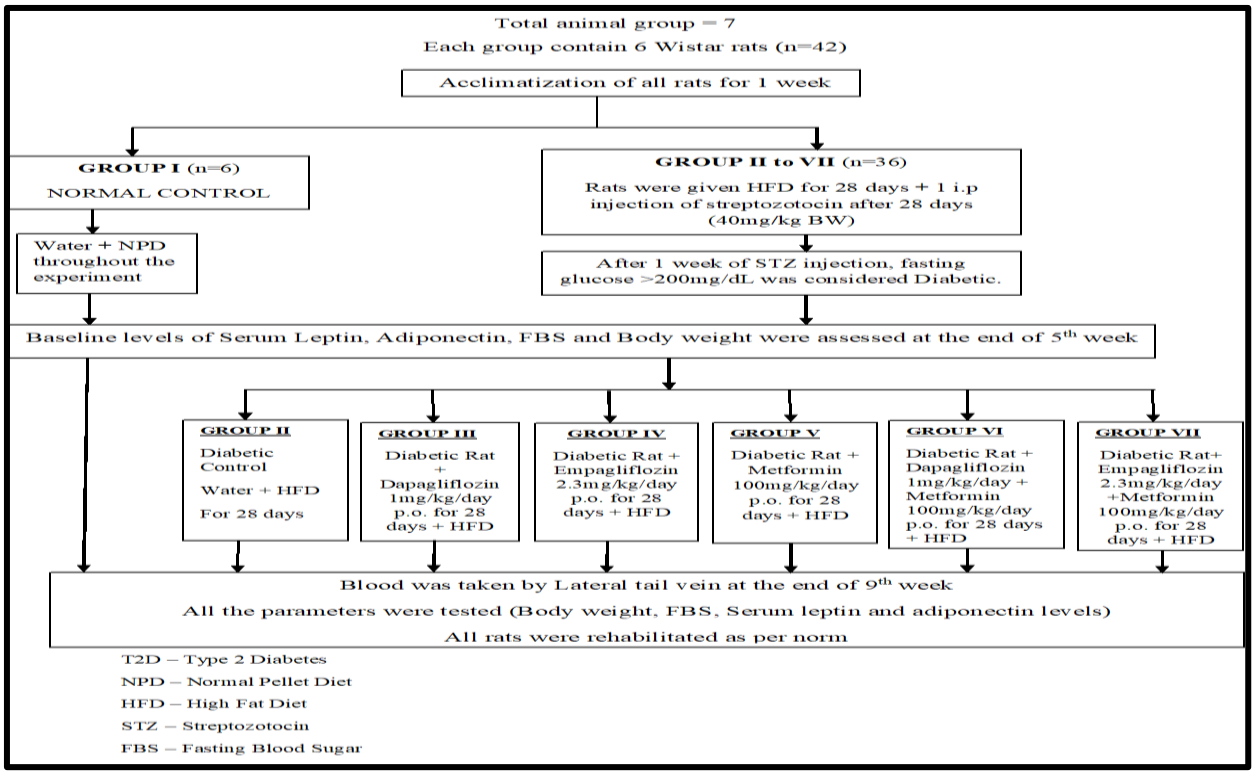
administered at a dose of 40 mg/kg BW. STZ at a dose of 40 mg/kg was prepared in cold citrate buffer (pH 4.5, 0.1 M)[20,21]**.** Rats were stabilized after one week of STZ injection. Following a week, blood samples were collected via the tail vein, and their blood glucose levels were measured using a glucometer (AccuSure Simple). Rats with blood glucose levels>200 mg/dL were considered diabetic rats and used in the study[22]**.**

**Experimental design**

A total of 42 male Wistar rats were enrolled in the research. They were randomized to seven groups (n=6 in each group) as follows: The study comprised seven groups: the Normal Control Group (Group I), the Untreated Diabetic Control Group (Group II), and the diabetic rats in Group III, who were administered Dapagliflozin at a dosage of 1mg/kg/day[23,24], diabetic rats in Group IV were treated with Empagliflozin (2.3 mg/kg/day)[24], diabetic rats in Group V were treated with Metformin (100 mg/kg/day)[24,25]**,** diabetic rats in Group VI were treated with a combination of DAPA(1mg/kg/day) and MET (100 mg/kg/day), diabetic rats in Group VII were treated with EMPA (2.3 mg/kg/day) and MET (100 mg/kg/day). All the drugs were administered orally after dissolving them in normal saline for 28 days after seven days after administration of STZ injection[26-28]. Group I and Group II were administered only 1 ml of normal saline p.o.

Baseline and final readings for serum leptin, serum adiponectin, blood glucose, and body weight were taken at five and nine weeks after the commencement of the study respectively.

**Flow chart of Experimental Protocol**




**Procedure**

**Blood Collection -** Blood samples were drawn from all the rats after five weeks, for baseline readings of serum leptin, serum adiponectin, blood glucose, and body weight. Samples were collected using the tail vein method and the body weight of each rat was measured. The rats were placed in a restrainer and tails were dipped into the container having lukewarm water to cause dilatation of the vein. The tail was gently wiped using gauze soaked in alcohol, enhancing the visibility of the lateral tail vein. Positioning the needle with the bevel facing upwards and nearly parallel to the vein, it was carefully inserted into the tail vein. Negative pressure was applied to the plunger. If the needle was in the vein flash of blood would be seen in the hub of the needle, and then blood was withdrawn. Blood collected was used to assess serum leptin and adiponectin, blood glucose levels at baseline, and at the end of the study as mentioned in the research methodology.

**Parameters measured**

**Measurement of body weight -** The rat's body weight was observed on a digital weighing machine available in Institutional Animal House. All the rats were properly marked and weighed.

**Estimation of Blood Glucose -** A blood sample was taken from the tail vein and utilized for blood glucose measurements. It was performed using a glucometer (AccuSure) with a small blood droplet.

**Estimation of Serum Leptin -** For quantitative measurement of serum leptin, we used the Rat Leptin Elisa

Kit procured from USCN Business Co., Ltd., and the assay procedure was followed as per the instruction manual provided along with the kit.

**Estimation of Serum Adiponectin -** For quantitative measurement of serum adiponectin, we used the Rat Adiponectin Elisa Kit procured from USCN Business Co., Ltd., and the assay procedure was followed as per the instruction manual provided along with the kit.

**Statistical Analysis**

The collected data was organized and tabulated in Microsoft Excel (Microsoft Office 365) and statistical analysis was done using SPSS version 23.0. The values were expressed as mean ± standard deviation. The change in group means at the two-time intervals was calculated using the paired t-test. Intergroup variations were assessed employing one-way ANOVA, followed by subsequent post-hoc analysis (Tukey-HSD test), was employed to assess individual group arrangements among others. p-value of less than 0.05 was considered statistically significant.

## RESULTS AND OBSERVATIONS

In this study, 42 rats were randomly allocated to seven study groups and were studied to evaluate the effect of Dapagliflozin, Empagliflozin, Metformin, and their two-drug (SGLT-2 inhibitor + Biguanide) combination therapy on Blood glucose, Body Weight, Serum Leptin & Adiponectin levels in streptozotocin-induced diabetic rats (Table 1).

**Intergroup comparison of Weight (gram) in the 5th & 9th week**

In 5th week, a significant variance in mean weight was observed in mean weight among normal rats (Group I) and diabetic rats (Group II-VII) ($p<0.001$). DAPA (-23.33 ± 7.2 gm), EMPA (-16.33 ± 17.18 gm), MET (-26.5 ± 1.38 gm), DAPA + MET (-51.33 ± 2.66 gm) and EMPA + MET (-40.5 ± 1.05 gm) groups showed a mean reduction in weight at 9$^{th}$ week as compared to baseline readings (5$^{th}$ week) while, Diabetic control showed an increase in weight by (5.17 ± 1.94 gm), at the end of 9$^{th}$ weeks. There was no significant weight gain seen in Group I (194 ± 12.69 gm). The highest difference was noted in Group VI whereas Group III, V & VII observed a significant weight reduction (Figure 1).

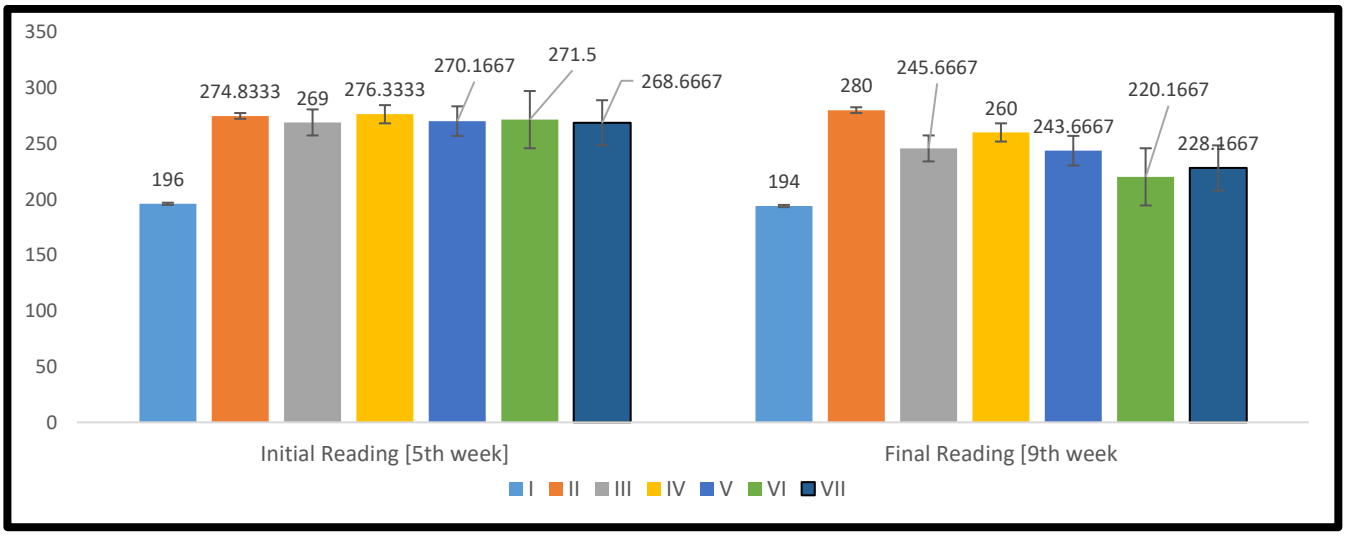



**Figure 1:** Intergroup comparison of Weight (gram) at 5th & 9th week

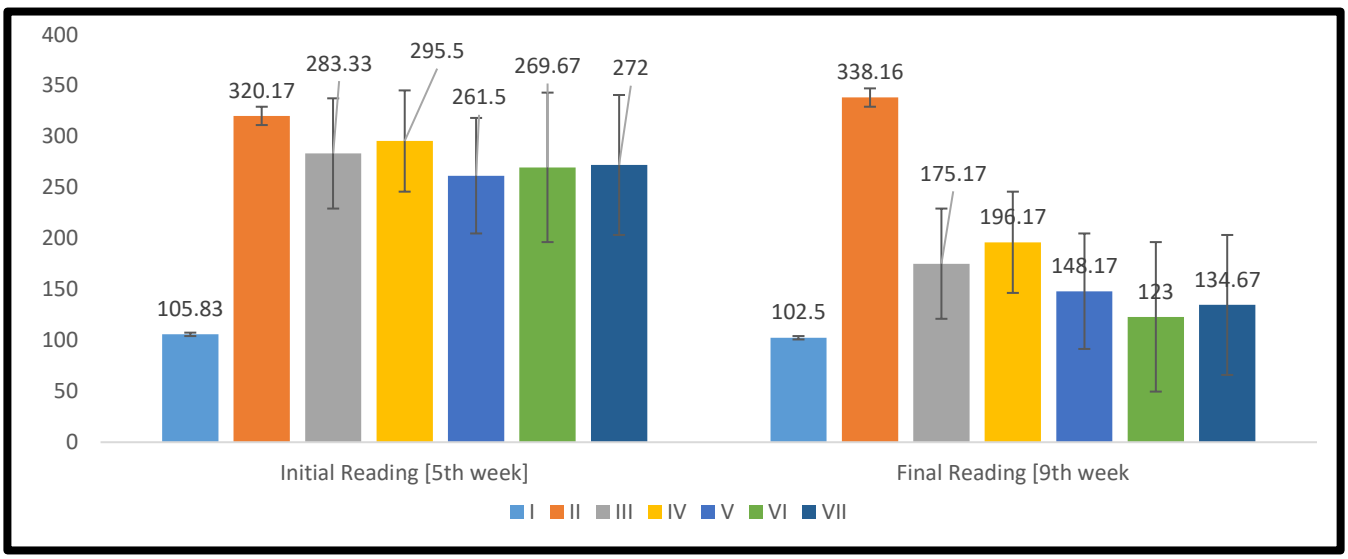



**Figure 2:** Intergroup Comparison of Blood glucose (mg/dL) at 5$^{th}$ & 9$^{th}$ week

**Table 1:** Effect of Dapagliflozin, Empagliflozin, Metformin, and their two-drug (SGLT-2 inhibitor + Biguanide) combination therapy on Blood glucose, Body Weight, Serum Leptin & Adiponectin levels in streptozotocin-induced diabetic rats.

| Group | Weight (gram) | | Blood glucose (mg/dl) | | Serum Leptin (ng/ml) | | Serum Adiponectin (ng/ml) | |
|---|---|---|---|---|---|---|---|---|
| | 5th week (Mean/ SD) | 9th week (Mean/ SD) | 5th week (Mean/ SD) | 9th week (Mean/ SD) | 5th week (Mean/ SD) | 9th week (Mean/ SD) | 5th week (Mean/ SD) | 9th week (Mean/ SD) |
| I [Normal control] | 196 ± 18.06 | 194 ± 12.69 | 105.83 ± 8.11 | 102.5 ±8.92 | 1.36 ± 0.21 | 1.56 ± 0.35 | 6.42 ± 0.67 | 6.33 ± 0.77 |
| II [Diabetic control] | 274.83 ± 30.85 | 280 ± 30.78 | 320.17 ± 35.14 | 338.16 ± 35.11 | 5.83 ± 0.78 | 6.23 ±0.92 | 1.68 ± 0.3 | 1.53 ± 0.37 |
| III [Dapagliflozin] | 269 ±17.56 | 245.66 ± 17.84 | 283.33 ± 56.35 | 175.17 ± 42.98 | 5.5 ± 0.62 | 3.12 ± 0.55 | 1.63 ±0.29 | 4.31 ± 0.24 |
| IV [Empagliflozin] | 276.33 ± 22.85 | 260 ± 16.85 | 295.5 ± 75.35 | 196.17 ± 60.91 | 5.14 ± 0.65 | 3.95 ± 0.53 | 1.43 ± 0.57 | 3.34 ± 0.37 |
| V [Metformin] | 270.16 ± 27.14 | 243.66 ± 26.37 | 261.5 ± 49.31 | 148.17 ± 50.61 | 7.17 ± 2.17 | 4.31 ± 1.62 | 1.8 ± 0.64 | 4.7 ± 0.67 |
| VI [Dapagliflozin + Metformin] | 271.5 ± 40.74 | 220.16 ± 39.23 | 269.67 ± 55.19 | 123 ± 54.80 | 6.14 ± 0.68 | 2.03 ± 0.26 | 1.67 ± 0.6 | 6 ± 1.07 |
| VII [Empagliflozin + Metformin] | 268.66 ± 34.49 | 228.16 ± 34.26 | 272 ± 53.7 | 134.67 ± 51.38 | 6.25 ± 0.93 | 2.89 ± 0.86 | 1.68 ± 0.77 | 4.85 ± 0.73 |

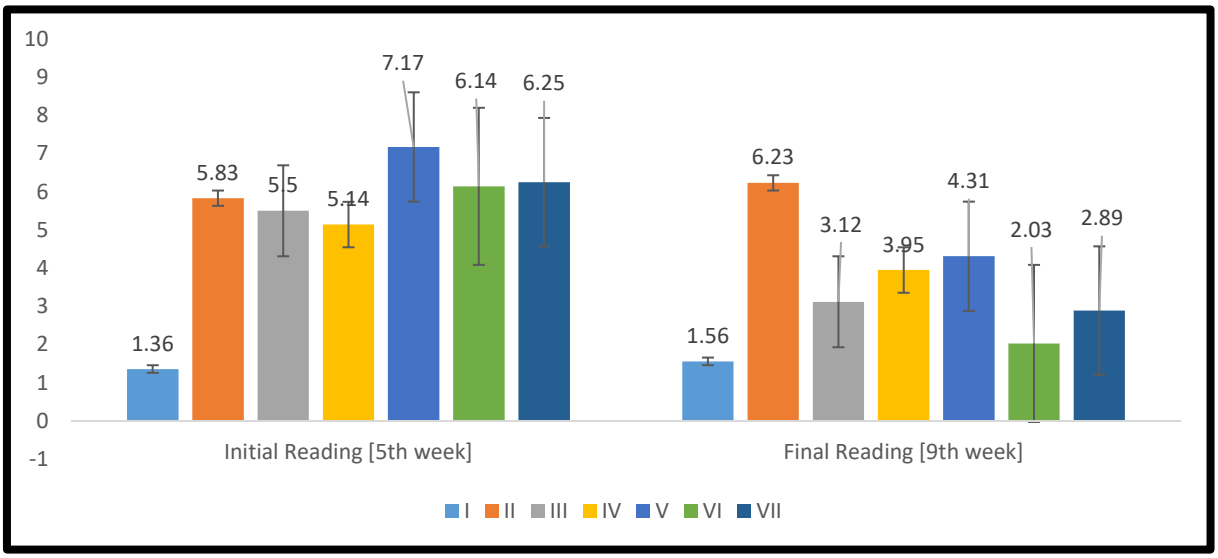



**Figure 3:** Intergroup comparison of Serum Leptin (ng/ml) at 5th & 9th week

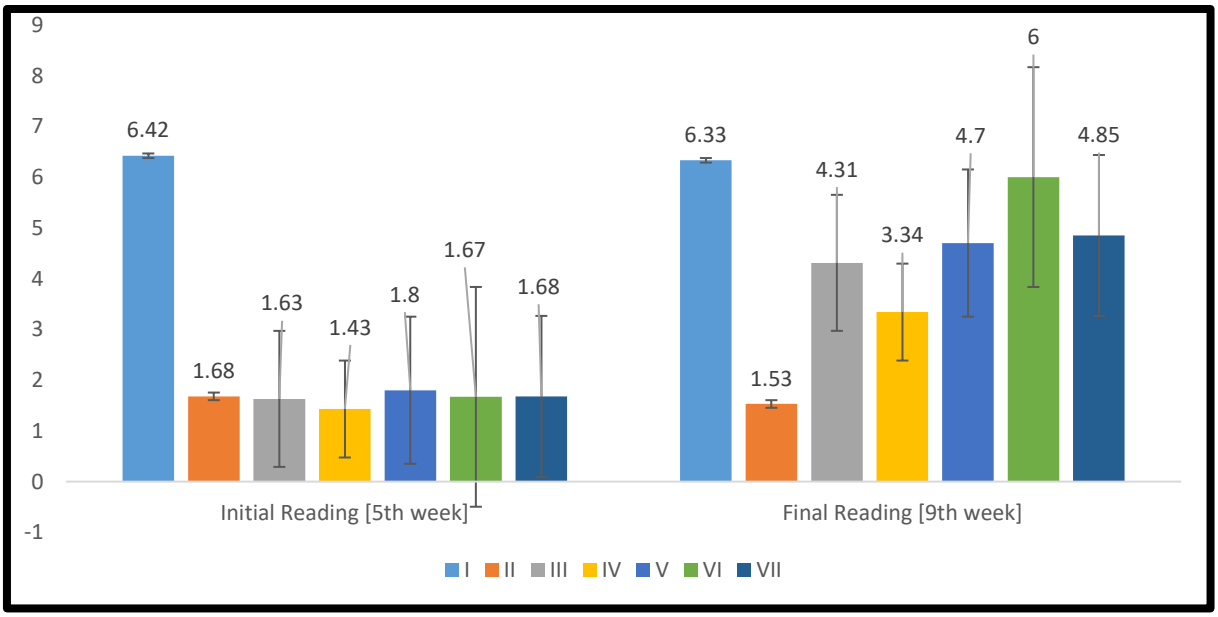



**Figure 4:** Intergroup comparison of Serum Adiponectin (ng/ml) at 5th & 9th week

**Intergroup Comparison of Blood glucose (mg/dL) at 5th & 9th week**

In the 5th week, a significant variance difference in mean blood glucose levels among normal rats (Group I) and diabetic rats (Group II-VII) ($p<0.001$). A mean rise in blood glucose was noted in Diabetic Control i.e., Group II by (18 ± 1.41) at the end of 9th week while in groups DAPA (-108.17 ± 14.61), EMPA (-99.33 ± 15.32), MET (-113.33 ± 12.26), DAPA + MET (-146.67 ± 1.86) and EMPA + MET (-137.33 ± 4.76) demonstrated a statistically significant drop in blood glucose level at the end of 9th week as compared to baseline readings (5th week). Combination therapy groups (Groups VI & VII) showed maximum blood glucose lowering effect as compared to monotherapy (Figure 2).

**Intergroup comparison of Serum Leptin (ng/ml) at 5th & 9th week**

In 5th week, there was a significant variance difference in mean serum leptin level among normal rats (Group I) and diabetic rats (Group II-VII) ($p<0.001$). A mean increase in serum leptin was noted in Diabetic Control i.e., Group II by (0.4 ± 0.49) at the end of 9th week while in groups DAPA (-2.38 ± 0.44), EMPA (-1.19 ± 0.15), MET (-2.86 ± 0.72), DAPA + MET (-4.11 ± 0.8) and EMPA + MET (-3.36 ± 0.36) demonstrated a significant decrease in serum leptin level at the end of 9th week in comparison to baseline readings (5th week). Combination therapy groups (Groups VI & VII) showed maximum reduction in serum leptin levels as compared to monotherapy (Figure 3)

**Intergroup comparison of Serum Adiponectin (ng/ml) at 5th & 9th week**

In 5th week, there was a significant variance difference in mean serum adiponectin levels among normal rats (Group I) and diabetic rats (Group II-VII) ($p<0.001$). A mean decline in serum adiponectin was noted in Diabetic Control i.e., Group II by (-0.15 ± 0.35) at the end of the 9th week while in groups DAPA (2.68 ± 0.18), EMPA (1.92 ± 0.6), MET (2.9 ± 0.48), DAPA + MET (4.33 ± 0.83) and EMPA + MET (3.17 ± 0.8) demonstrated a significant increase in serum adiponectin level at the end of 9th week in comparison to baseline readings (5th week). Combination therapy groups (Groups VI & VII) showed a maximum increase in serum adiponectin levels as compared to monotherapy (Figure 4)

## DISCUSSION

Diabetes, once thought of as a single disease entity, is now understood to be a heterogeneous group of illnesses, each of which is characterized by a state of chronic hyperglycaemia brought on by a variety of environmental and genetic factors acting in conjunction. About 90% to 95% of diabetic cases are T2DM, with the largest prevalence rates occurring in low- and middle-income nations[8,29].

Approximately 80% of type 2 DM patients have obesity, the majority of which is visceral or central (as indicated by the hip-to-waist ratio)[8]. Over 1 billion people worldwide are expected to be living with obesity (BMI 30kg/m$^2$) by 2030. If we are to tackle preventable NCDs, then success in addressing high BMI is essential[30].

There is a need for such pharmacological agents that not only prevent or treat hyperglycaemia but also cause weight loss, decrease chronic inflammation, and prevent cardiovascular complications, which is one of the primary causes of mortality in T2DM patients in countries like India.

According to most guidelines, metformin stands as the initial treatment option for type 2 diabetes and has also been shown to stop the development of Impaired glucose tolerance into diabetes.

The question arises, when the desired effect is not achieved by metformin alone, then which pharmacological agent should be added?

In routine clinical practices insulin secretagogues are preferred which causes a risk of weight gain and hypoglycaemia but newer drugs like SGLT-2 inhibitors due to their unique mechanism of action cause glucosuria and control fasting as well as post-prandial blood glucose independent of insulin action. These medications also have negligible risks of hypoglycaemia since they do not increase the secretion of insulin. Weight loss may also be aided by glucose-based calorie loss in the urine. Additionally, osmotic diuresis brought on by glycosuria may be beneficial for patients with hypertension and fluid overload[7,9].

Furthermore, SGLT-2 inhibitors are proposed as a means to restore the balance between pro- and anti-inflammatory adipokines[10].

In the present study, we tried to evaluate the role of Dapagliflozin, Empagliflozin, Metformin, and their two-drug (SGLT-2 inhibitor + Biguanide) combination therapy on Blood glucose, Body Weight, Serum Leptin & Adiponectin levels in streptozotocin-induced diabetic rats.

**Comparison of weight changes among therapeutic groups (monotherapy and combination therapy) and controls**

At week 5 diabetic control (Group II) and all the groups (III to VII) showed significant variance in weight as compared to the normal control (Group I). No significant weight differences were noted between rats in groups (III to VII) and diabetic control group (II) at week 5. However, the rats in combination therapy groups (VI and VII) were found to have statistically significant differences as compared to the diabetic control group (II) at week 9 with values similar to Normal control group rats.

**Saad *et al.* (2015)** studied that Metformin administration resulted in weight loss at the end of the 4-week treatment period, however, the change was not statistically significant as compared to the control group[31].

**Comparison of Blood glucose levels among therapeutic groups (monotherapy and combination therapy) and controls**

At week 5 diabetic control (II) and all the groups (III to VII) showed statistically significant change in blood glucose in comparison to the normal control (I) group.

No significant blood glucose differences were noted between rats in groups (III to VII) and diabetic control group (II) at week 5. However, all the therapeutic groups (III to VII) showed a statistically significant decrease in mean blood glucose levels in comparison to the diabetic control group (II) at week 9. Thus, both monotherapy and combination therapy showed evident action in lowering blood glucose levels as compared to the diabetic control group. The results suggest that both SGLT-2 inhibitors and Biguanide and their combination affected reducing the blood glucose levels.

**Dhillon (2019)** reviewed literature related to SGLT-2 inhibitor Dapagliflozin and its role in T2DM. It noted that numerous clinical studies have reported effective glycaemic control action of Dapagliflozin used as a standalone therapy and in combination with other anti-hyperglycaemic agents[9]. Our findings are also consistent with this reporting as far as blood glucose control is concerned.

**Saad *et al.* (2015)** studied that Metformin administration resulted in a time-dependence reduction in blood glucose, and the rats in the treatment group had significantly low level of blood glucose levels than the rats in untreated groups. Their study included anti-diabetic drugs metformin, glimepiride, and sitagliptin[31].

**Comparison of Serum Leptin levels among therapeutic groups (monotherapy and combination therapy) and controls**

At week 5 diabetic control (Group II) and all the groups (III to VII) showed statistically significant change in mean Serum Leptin levels in comparison to the normal control (I) group. No significant changes in mean Serum Leptin levels were noted between rats in groups (III to VII) and diabetic control group (II) at week 5. However, all the therapeutic groups (III to VII) showed a statistically significant reduction in mean Serum Leptin levels as compared to the diabetic control group (II) at week 9.

Tukey HSD test reports, significant differences in mean Serum Leptin levels for combination therapy of Metformin + Dapagliflozin group (VI) when compared with monotherapy groups involving Empagliflozin (IV) and Metformin (V) at week 9.

The mean serum leptin levels were 2.03 ± 0.26 ng/ml in Metformin + Dapagliflozin treated group (Group VI) as compared to 3.95 ± 0.53 ng/ml in Empagliflozin treated group (Group IV) and 4.31 ± 1.62 ng/ml in Metformin treated group (Group V) at week-9, respectively. The mean serum leptin level was 2.89 ± 0.86 ng/ml for Metformin + Empagliflozin treated group (Group VII), which is also lower as compared to monotherapy groups (III to V). Thus, Metformin and SGLT-2 inhibitors combination therapy had a significant effect on Serum Leptin levels as compared to monotherapies involving either Metformin or any of the SGLT-2 inhibitors used in the study.

**Muoio *et al.* (2008)** reported that T2DM is associated with increased serum leptin levels due to impaired leptin signalling and action resulting from the state of leptin resistance[32]. Our study illustrated that all the investigated drugs led to a reduction in serum leptin levels, however, the combination therapies were more effective as compared to the monotherapies. The results suggest that the drugs included in our study could enhance leptin sensitivity and correct leptin resistance in T2DM.

**Matsui *et. al.* (2010)** In their study, it was noted that a majority of obese individuals exhibit hyperleptinemia, which is associated with leptin resistance. As a result, decreasing in body weight will improve leptin and insulin resistance. In this study, metformin treatment improved hyperinsulinemia and hyperleptinemia as well as hyperglycemia[33].

In our study also Metformin treated group (Group V) decreased circulating leptin levels, and their combination with SGLT-2 inhibitors had an additive effect. Thus, our findings are consistent with published literature.

**Comparison of Serum Adiponectin levels among therapeutic groups (monotherapy and combination therapy) and controls**

At week 5 diabetic control (Group II) and all the groups (III to VII) showed statistically significant change in mean Serum Adiponectin levels in comparison to the normal control (Group I). No significant variance in mean Serum Adiponectin levels was noted among rats in groups (III to VII) and diabetic control groups (Group II) at week 5. However, all the therapeutic groups (III to VII) showed a statistically significant increase in mean Serum Adiponectin levels in comparison to the diabetic control group (Group II) at week 9.

Tukey HSD test report, mean serum adiponectin levels were higher 6.00 ± 1.07 ng/ml in Metformin + Dapagliflozin treated group (Group VI) as compared to 4.31 ± 0.24 ng/ml, 3.34 ± 0.37 ng/ml and 4.70 ± 0.67 ng/ml for Dapagliflozin (III), Empagliflozin (IV) and Metformin (V) treated groups at week-9, respectively.

The mean serum adiponectin level was 4.85 ± 0.73 ng/ml for Metformin + Empagliflozin treated group (Group VII), which is also higher as compared to monotherapy groups (III to V), but it showed a statistically significant difference only as compared to Empagliflozin treated group (Group IV) at week-9. Thus, Metformin and SGLT-2 inhibitors (Dapagliflozin / Empagliflozin) combination therapy had a significant effect on Serum Adiponectin levels as compared to monotherapies involving either Metformin or any of SGLT-2 inhibitors.

**Bailey *et. al.* (2012)** delineated the glucose-lowering and weight-reducing effectiveness of dapagliflozin when used

as a sole therapy in individuals newly diagnosed with type 2 diabetes. Certainly, the decline in waist circumference observed in this study aligns with the enhancements noticed in leptin and adiponectin levels among the dapagliflozin groups[34]**.** In our study also, SGLT-2 inhibitors increased the serum adiponectin levels in T2DM.

**Kashiwagi *et. al.* (2014**) studied those individuals who received ipragliflozin as an adjunct treatment following metformin and experienced reductions in body weight and waist circumference, along with an elevation in adiponectin levels[35]. Our study demonstrated that all drugs under investigation increased serum adiponectin levels, however, the combination therapies were more effective as compared to the monotherapies. The elevated serum adiponectin could aid a possible mechanism for the beneficial cardiovascular effects of anti-diabetic drugs.

## CONCLUSION

This study concluded that the addition of SGLT-2 inhibitors to metformin for diabetes treatment led to notable enhancements in glycaemic regulation, lowered body weight, and decreased insulin resistance. These effects were attributed to reducing oxidative stress by modulation of adipokine levels i.e., decrease in pro-inflammatory cytokine - serum leptin and increase in insulin-sensitizing anti-inflammatory cytokine - serum adiponectin.

The beneficial effect of Dapagliflozin and Empagliflozin can additionally be associated with their ability to reverse insulin resistance by alleviating the state of leptin resistance. Therefore, the combination of SGLT-2 inhibitors and metformin emerges as a promising strategy for managing T2DM patients with obesity.

This study provides an idea regarding the impact of SGLT-2 inhibitors in combination with metformin in animal diabetic model. It still leaves a lacuna to explore the understanding and effect of other anti-diabetic drugs and their combinations in modulating adipokine levels or other similar biomarkers indicating insulin resistance in T2DM patients. This can pave the way for improving existing prescription practices for the treatment of a metabolic disorder like diabetes.

### ACKNOWLEDGEMENTS

The authors are thankful to Dr. S.C Sharma, Dr. Fagni Garg, and Dr. Shreedhar Angadi for providing continuous support during the study.

### DECLARATIONS

***Ethical approval:*** Approved. Compliance with Ethics Guidelines: All procedures performed in studies involving animals were by the ethical standards of the Institutional Animals Ethics Committee as per the guidelines laid down by the Committee for Control and Supervision of Experiments on Animals (CCSEA).

**Source of Support:** The author(s) received no financial support for the research, authorship, and/or publication of this article

**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## REFERENCES

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol 2018;14:88–98. https://doi.org/10.1038/nrendo.2017.151.
2. Diabetes Mellitus - StatPearls - NCBI Bookshelf n.d. https://www.ncbi.nlm.nih.gov/books/NBK551501/ (accessed December 11, 2022).
3. IDF. IDF Diabetes Atlas 2021. 2021.
4. Pradeepa R, Ophthalmology VM-IJ of, 2021 undefined. Epidemiology of type 2 diabetes in India. NcbiNlmNihGov n.d.
5. https://idf.org/our-network/regions-and-members/south-east-asia/members/india/ (accessed December 12, 2023).
6. Papadakis MA, McPHEE SJ, Rabow MW. Current medical diagnosis & treatment. Diabetes Mellitus & Hypoglycemia. 61st Edition. McQuaid KR, editor. McGraw Hill; 2022. p. 1212-1218.
7. Saleem F. Dapagliflozin: Cardiovascular Safety and Benefits in Type 2 Diabetes Mellitus. Cureus 2017;9. https://doi.org/10.7759/cureus.1751.
8. Loscalzo J, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. Obesity, Diabetes Mellitus, and Metabolic Syndrome. 21st Edition. McGraw Hill; 2022. VOLUME 1. p. 3080-3150.
9. Dhillon S. Dapagliflozin: A Review in Type 2 Diabetes. Drugs 2019;79:1135–46. https://doi.org/10.1007/s40265-019-01148-3.
10. Tentolouris A, Vlachakis P, Tzeravini E, Eleftheriadou I, Tentolouris N. SGLT2 inhibitors: A review of their antidiabetic and cardioprotective effects. Int J Environ Res Public Health 2019;16:1–27. https://doi.org/10.3390/ijerph16162965.
11. Katsiki N, Mikhailidis DP, Banach M. Leptin, cardiovascular diseases and type 2 diabetes mellitus review-article. Acta Pharmacol Sin 2018;39:1176–88. https://doi.org/10.1038/aps.2018.40.
12. Abdella NA, Mojiminiyi OA. Clinical applications of adiponectin measurements in type 2 diabetes mellitus: Screening, diagnosis, and marker of diabetes control. Dis Markers 2018;2018. https://doi.org/10.1155/2018/5187940.
13. Manual A. Dietary Guidelines for Indians - A Manual. Natl Inst Nutr Hyderabad 1998;500.
14. Misra A, Sharma R, Gulati S, Joshi SR, Sharma V, Ghafoorunissa, et al. Consensus dietary guidelines for healthy living and prevention of obesity, the metabolic syndrome, diabetes, and related disorders in Asian Indians. Diabetes Technol Ther 2011;13:683–94. https://doi.org/10.1089/dia.2010.0198.
15. Steiner S. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. Zeitschrift Fur Gefassmedizin 2016;13:17–8. https://doi.org/10.1056/nejmoa1504720.
16. Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, et al. Metformin suppresses gluconeogenesis by

inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 2014;510:542–6. https://doi.org/10.1038/nature13270.

17. Aubert G, Mansuy V, Voirol MJ, Pellerin L, Pralong FP. The anorexigenic effects of metformin involve increases in hypothalamic leptin receptor expression. Metabolism 2011;60:327–34. https://doi.org/10.1016/j.metabol.2010.02.007.
18. Tang X, Li J, Xiang W, Cui Y, Xie B, Wang X, et al. Accepted Preprint first posted on 10 June 2016 as Manuscript JOE-16-0142 Title page Metformin Increases Hepatic Leptin Receptor and Deceases steatosis in Mice Short title : Word count : 4050 Abstract. 2016.
19. Gheibi S, Kashfi K, Ghasemi A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed Pharmacother 2017;95:605–13. https://doi.org/10.1016/j.biopha.2017.08.098.
20. De MagalhÃes DA, Kume WT, Correia FS, Queiroz TS, Allebrandt Neto EW, Dos Santos MP, et al. High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: A new proposal. An Acad Bras Cienc 2019;91:1–14. https://doi.org/10.1590/0001-3765201920180314.
21. Motyl K, McCabe LR. Streptozotocin, type i diabetes severity and bone. Biol Proced Online 2009;11:296–315. https://doi.org/10.1007/s12575-009-9000-5.
22. Qinna NA, Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. Drug Des Devel Ther 2015;9:2515–25. https://doi.org/10.2147/DDDT.S79885.
23. Gamil NM, Abd El Fattah MA, Ahmed MAE, Maklad YA, Gamal el Din AA, Eid NI. Lansoprazole enhances the antidiabetic effect of dapagliflozin in fortified diet-fed streptozotocin-treated diabetic rats. J Biochem Mol Toxicol 2020;34. https://doi.org/10.1002/jbt.22451.
24. Medhi B, Prakash A. Practical Manual of Experimental and Clinical Pharmacology. 2nd Edition. Jaypee Brothers Medical Publishers; 2017. p. 27-29.
25. Bakhteyari A, Nikpour P, Mostafavi FS, Eskandari N, Matinfar M, Asl SS, et al. Impact of metformin and pioglitazone on serum level of tumor necrosis factor-alpha and lipid profiles during implantation window in diabetic rats. Int J Fertil Steril 2019;13:148–53. https://doi.org/10.22074/ijfs.2019.5636.
26. Jaikumkao K, Pongchaidecha A, Chueakula N, Thongnak L, Wanchai K, Chatsudthipong V, et al. Renal outcomes with sodium glucose cotransporter 2 (SGLT2) inhibitor, dapagliflozin, in obese insulin-resistant model. Biochim Biophys Acta - Mol Basis Dis 2018;1864:2021–33. https://doi.org/10.1016/j.bbadis.2018.03.017.
27. M. Kabel A. Targeting Apoptosis, Advanced Glycation End Products and Transforming Growth Factor-β1 by Biguanides and Resveratrol to Ameliorate Experimentally-induced Diabetic Nephropathy. J Drug 2015;1:1–9. https://doi.org/10.24218/jod.2015.1.
28. Lee TI, Chen YC, Lin YK, Chung CC, Lu YY, Kao YH, et al. Empagliflozin attenuates myocardial sodium and calcium dysregulation and reverses cardiac remodeling in streptozotocin-induced diabetic rats. Int J Mol Sci 2019;20. https://doi.org/10.3390/ijms20071680.
29. World Health Organisation. Classification of diabetes mellitus-2019. n.d.
30. The World Obesity Federation. Projections of Obesity Prevalence in 2030. World Obes Atlas 2022 2022:18–41.
31. Saad MI, Kamel MA, Hanafi MY. Modulation of adipocytokines production and serum NEFA level by metformin, glimepiride, and sitagliptin in HFD/STZ diabetic rats. Biochem Res Int 2015;2015. https://doi.org/10.1155/2015/138134.
32. Muoio DM, Newgard CB. Mechanisms of disease: Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:193–205. https://doi.org/10.1038/nrm2327.
33. Matsui Y, Hirasawa Y, Sugiura T, Toyoshi T, Kyuki K, Ito M. Metformin reduces body weight gain and improves glucose intolerance in high-fat diet-fed C57BL/6J Mice. Biol Pharm Bull 2010;33:963–70. https://doi.org/10.1248/bpb.33.963.
34. Bailey C, Iqbal N, Diabetes CT-, and O, 2012 undefined. Dapagliflozin monotherapy in drug-naive patients with diabetes: a randomized-controlled trial of low-dose range. Wiley Online Libr n.d.
35. Kashiwagi A, Kazuta K, Goto K, Yoshida S, Ueyama E, Utsuno A. Ipragliflozin in combination with metformin for the treatment of Japanese patients with type 2 diabetes: ILLUMINATE, a randomized, double-blind, placebo-controlled study. Diabetes, Obes Metab 2015;17:304–8. https://doi.org/10.1111/dom.12331.