ORIGINAL ARTICLE

EFFECTS OF WALNUT LEAVES EXTRACT ON EFFICACY OF DAPAGLIFLOZIN IN TYPE II DIABETIC MICE

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Background: With a rising prevalence of type 2 diabetes mellitus its becoming more important than ever to find new strategies to prevent and cure the disease. Objective of this study was to assess the effects of walnut leaves extract on the efficacy of dapagliflozin in type 2 diabetic mice. **Methods:** It was experimental, randomized control study. A total of 50 male Balb/c mice were included in this study and randomly divided into two groups: Group 1 (Normal Control) consisting of 10 mice and Group 2 (experimental group) of 40 mice. The experimental group was further divided into 4 groups of 10 mice each following induction of type 2 diabetes: Group 2 (Disease Control), Group 3 (Ethanolic walnut leaves extract treated), Group 4 (Dapagliflozin treated) and Group 5 (Combination of dapagliflozin and ethanolic walnut leaves extract treated) for a duration of 45 days. Statistical analysis was done using SPSS-27. One-way ANOVA (post hoc Tukey test) was used to compare the means of HbA1c among groups, and $p<0.05$ was considered significant. **Results:** Mice treated with walnut leaves extract (Group 3), dapagliflozin (Group 4), and the combination of walnut leaves extract and dapagliflozin (Group 5) exhibited significantly reduced serum HbA1c levels compared to those observed in the diabetic control group ($p<0.05$). **Conclusions:** The combined administration of walnut leaves extract and dapagliflozin leads to a significant decrease in serum HbA1c levels in diabetic mice, as compared to mice treated with walnut leaves extract or dapagliflozin alone.

Keywords: Diabetes Mellitus, Walnut leaf, Dapagliflozin, HbA1c

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INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, is an important issue for global health that affects many people.[1] In DM, the body's capacity to regulate glucose in the blood is compromised, marked by elevated blood glucose levels. Insulin contributes in control of blood glucose level. Type 1 Diabetes mellitus (T1DM) is caused by insufficient insulin production whereas resistance to insulin leads to type 2 diabetes mellitus (T2DM).[2]

Obesity, lack of physical exercise, genetic susceptibility, and advancing age are the primary risk factors for T2DM. According to the International Diabetes Federation (IDF), prevalence DM has reached alarming levels throughout the world as it has affected roughly 463 million people aged 20–79 years in 2019. T2DM accounts for over 90% of all these diabetic cases. If certain precautionary measures are not taken the current trends might continue to rise and number of the patients might reach 700 million by the year 2045.[3] DM has become a significant public health concern in Pakistan, affecting 19.4 million individuals. According to recent estimates by IDF, over 33,000,000 people in Pakistan have T2DM. This illness affects around 26.8% of adults, emphasizing the critical need for effective preventative and management techniques. The estimated number of people in the nation with T2DM will be 26.1 million by 2030 showing a considerable increase in disease prevalence.[4] Due to its complex metabolic makeup, DM demands thorough and detailed medical care and attention. Despite the medical treatment, patients must actively seek healthy diet and exercise plans to deal with this disease. Therefore, diabetes therapy must include oral hypoglycaemic medicines and lifestyle changes.[5] Pharmaceutical treatment options now accessible for T2DM include metformin, sulfonylureas, thiazolidinediones, and sodium-glucose cotransporter-2 (SGLT2) inhibitors.[6] In recent years, there has been a notable increase in interest surrounding using SGLT2 inhibitors within the realm of oral hypoglycaemic medications.[7]

Dapagliflozin, a selective SGLT2 inhibitor, functions by inhibiting the activity of SGLT2 receptors located in the renal tubules, thereby leading to augmented renal glucose excretion. This approach is associated with the reduction of blood glucose levels and the promotion of weight loss. Dapagliflozin exhibits notable benefits, including its capacity to decrease HbA1c levels, enhance cardiovascular well-being and operate through an insulin-independent mechanism.[8]

There has been a notable increase in scholarly attention toward utilizing botanical substances for managing diabetes. Ayurveda and traditional Chinese medicine have acknowledged the potential of herbs in

managing diabetes. The glucose lowering effects of walnut leaves (*Juglans Regia L.*) have been the subject of scientific investigation. These leaves possess bioactive compounds that have the potential to enhance glucose metabolism and increase insulin sensitivity.[9] Use of walnut leaves as a potential therapeutic intervention for diabetes has been documented in the scientific literature.[10] The polyphenols and flavonoids found in walnut leaves have been shown to increase insulin sensitivity, stimulate glucose absorption by cells, and inhibit carbohydrate metabolizing enzymes.[11]

Both walnut leaves extract and dapagliflozin have established hypoglycaemic properties, but their combined and comparative effects have not been explored yet. This study was designed to observe these two aspects of walnut leaves extract. Adding walnut leave extract as an adjuvant with dapagliflozin may increase efficacy of this drug.

MATERIAL AND METHODS

This comparative experimental investigation was conducted at Animal House, Multidisciplinary Research Laboratory, and Pharmacology Laboratory of Islamic International Medical College (IIMC), Islamabad (Ref.#: Riphah/IRC/22/2081) after approval from the Ethical Review Committee. The duration of the study was 45 days from 1st April 2023 to 20th May2023. The study involved 50 healthy male albino Balb/c mice, aged 6–8 weeks and weighing 30–50 g. The mice were allowed free access to tap water. The standard feed was prepared at IIDC. The light and dark cycle followed a 12-hour pattern.

Walnut (*J. Regia*) leaves, sourced from the vicinity of Muzaffarabad, Azad Kashmir, dried in shade, and were grinded into powder. The 1,000 g of powdered *J. Regia* leaves were soaked in 70% ethanol for 24 hours, and extracted three times using new 96% ethanol for a total of 24 hours at room temperature.[12] The resultant solution was evaporated in a rotary evaporator at 55 °C after filtration through Whatmann #1 paper. The extracted material was kept in airtight glass bottle, shielded from light, and refrigerated at 2–8 °C.[13]

Following a week of acclimation, the mice were split into two groups at random: 10 mice in control Group 1 and 40 mice in experimental group. Group-1 was fed regular food for 5 days. The experimental group received a normal meal with intraperitoneal injections of streptozotocin (40 mg/Kg/day) for 5 days in a row.[14] The fasting blood glucose levels of Group-1 and experimental group were measured using Accuchek® instant glucometer, and compared confirming diabetes in the experimental group.

The experimental group was then split into Groups 2, 3, 4, and 5. The mice in group-2 were designated as Disease Control and were fed only standard chow diet. For 40 days, Group-3 mice were fed a standard chow diet enriched with 200 mg/Kg of ethanolic walnut leaf extract (WLE) each day.[15] The mice in Group-4 were given a regular chow diet combined with an oral dose of the medication Dapagliflozin (1 mg/Kg/day) in drinking water.[16] Group-5 mice were given a standard chow diet supplemented with 200 mg/Kg of ethanolic walnut leaf extract and 1 mg/Kg of dapagliflozin medication added into their drinking water. The final blood samples were completed after 40 days of treatment.

SPSS-27 was used for statistical analysis. The results were reported as Mean±SD. The quantitative parameters between the 5 groups were compared using one-way ANOVA (Post-hoc Tukey test), and $p<0.05$ was considered as significant.

RESULTS

The mean HbA1c (%) in Group-1 was 4.59±0.4122, and in Group-2 it was 7.87±0.34, and was considerably higher than Group-1 ($p<0.001$). In Group-3 the mean HbA1c was 5.20±0.63, it was (5.00±0.54) in Group-4, and 4.66±0.44 in Group-5. Mean of HbA1c (%) was considerably lower than that of Group-2 ($p<0.001$) in Group-3, 4, and 5. Table-1 presents a comparison of Mean±SD of HbA1c (%) in each group.

Table-1: HbA1c levels in all groups (Mean±SD)

Groups	HbA1c (%)	*p*
Group-1 (Normal control)	4.59±0.4122	<0.001*
Group-2 (Disease control)	7.87±0.3498	
Group-3 (WLE treated)	5.20±0.6394	
Group-4 (DAPA treated)	5.00±0.5466	
Group-5 (WLE+DAPA)	4.66±0.4458	

*Significant

DISCUSSION

Findings of the current study support the notion that all experimental drugs significantly reduce the hyperglycaemia caused by streptozotocin; nevertheless, the combined effects of dapagliflozin and walnut leaf extract show very promising outcomes.

In the present study T2DM was induced in all experimental mice by using streptozotocin (STZ) for 5 days intraperitoneally with resultant increase in fasting blood glucose levels. These findings are similar with results of Arulmozhi DK *et al*[14], Rato L, *et al*[17] and Wang J *et al*[18] also used same dose of STZ intraperitoneally in experimental animals for induction of T2DM and observed marked changes in insulin and blood glucose levels.

Group-3 in our study received WLE and the results revealed significant improvement in FBS levels and HbA1c in comparison to disease control group. Our findings are in agreement with Asgary S *et al*[19]. Outcomes of present study are in promise with the facts reported by Nasiry D *et al*[15] who performed a study on protective effects of methanolic WLE on STZ-induced

diabetic peripheral neuropathy in rats. In Group-4 marked reduction was observed in FBS levels and HbA1c in comparison to disease control group. Chang DY *et al*[20] had similar findings, who investigated the antidiabetic effects of dapagliflozin in type 2 diabetic mice and observed that dapagliflozin significantly decreased blood glucose levels and HbA1c. It was also Findings in Group-4 are supported by study of Wei R *et al*[16] who investigated the antidiabetic effects of dapagliflozin in type 2 diabetic mice and reported that dapagliflozin significantly decreased blood glucose and upregulated plasma insulin and GLP-1 levels by promoting beta cell regeneration, enhancing beta cell self-replication.

We observed that the antidiabetic effect was more marked in Group-5 compared to Group-3 and 4. Abdel-Wahab AF *et al*[21] demonstrated that dapagliflozin and irbesartan caused significant reduction in blood glucose levels and HbA1c levels when used alone and combination therapy also has remarkable protective effects on renal function and structure.

CONCLUSIONS

The antidiabetic effects of dapagliflozin were more marked compared to ethanolic walnut leaf extract in diabetic mice. The combined effect of walnut leaf extract and dapagliflozin was more pronounced as compared to using dapagliflozin or walnut leaf extract alone in managing high blood sugar in diabetic mice. Further work is recommended on the active ingredients of walnut leaves and their interactions with other drugs.

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