Full Length Research Article

Neuroprotective Effect of Troxerutin against Cognitive Impairment in Type 2 Diabetes Mellitus Mice Model

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ABSTRACT

Background: Type 2 diabetes (T2DM) is predominantly associated with obesity in people of all ages, both of which cause major worldwide morbidity and deaths. The present study evaluates the possible neuroprotective impact of troxerutin (TROX) in decreasing the cognitive impairment caused by T2DM.

Methods: Five groups of mice received different diets for nineteen weeks: a regular diet, TROX as a drug control group, diet rich in fat (HFD), streptozotocin (STZ) with HFD and HFD+STZ with TROX supplementation. The metabolic indicators such as daily food consumption, weekly body weight, body mass index and blood glucose level were assessed. Other data points observed in week 19 included insulin and glucose tolerance, fasting blood glucose and behavioral evaluations. In brain homogenates and tissues the ROS level and antioxidant enzyme activities were assessed.

Results: The body weight of the HFD group increased significantly ($P < .0001$) compared to the control, drug control and HFD+STZ groups. Additionally, the HFD+STZ+TROX group had decreased the daily food consumption ($P = .0036$), enhanced body weight ($P < .0001$), adipose tissue weight ($P = .0009$), insulin/glucose tolerance ($P < .0001$) and reduced glucose/fasting blood levels ($P < .0001$) versus the HFD+STZ group. In comparison to the control group, the treated group, demonstrated a significant increase in food intake ($P < .0001$) as well as in body weight ($P < .0001$). Furthermore, the TROX therapy improved anxiety symptoms and cognitive performance. Reduced antioxidant enzymes activities and a considerable enhancement in lipid peroxidation were observed in HFD+STZ group. The TROX treatment reduced these adverse effects by lowering lipid peroxidation and ROS level ($P < .0001$) in mice brain.

Conclusions: According to these outcomes, cognitive impairment, oxidative imbalance and metabolic stress are all brought on by T2DM. On the other hand, TROX efficiently reverses these effects, possibly due to its antioxidant qualities, which improve brain function and provide neuroprotection.

INTRODUCTION

The metabolic condition known as diabetes mellitus is distinguished by consistently high blood sugar levels that are caused by either insufficient insulin synthesis, insulin resistance or both [1]. Ninety percent of cases of diabetes are T2DM, which is marked as insulin resistance. It is a worldwide most frequent and clinical important pathophysiological condition and its prevalence has become a serious public health concern [2]. The International Diabetes Federation predicts that by 2045, over 0.783 billion people, or one in eight individuals would be living with diabetes [3]. Both advanced and developing nations, including Pakistan are seeing a sharp rise in the prevalence of T2DM. According to estimates, the number of adult diabetic in Pakistan increased from 5.2 million in 2000 to over 33 million by 2021 [4,5].

Despite being more frequent in those over 45, T2DM is becoming more common in younger people as a result of increased rates of obesity, inactivity and diets high in calories [6]. Major complications from T2DM include impaired vision, nerve disease, damaged kidneys and coronary artery disease. Due to its substantial association with the risk of dementia and cognitive decline, its effect on the brain is widely recognized [7]. T2DM may account for one in every ten instances of dementia globally and also doubled the long term dementia risk in patients [7].

It is essential to highlight that in many situations the traditional medications are ineffective to provide long-term glucose control. Currently, better eating habits and more physical exercises are the best ways to stop or prevent T2DM. In this context, the naturally occurring substances present in fruits and vegetables called flavonoids have attracted a lot of research due to their non-toxic nature and potential for use as very strong chemo preventive agent against obesity and T2DM [8,9]. Flavonoids have also a strong antioxidant and anti-inflammatory activities [10].

Troxerutin (sometimes called vitamin P4) is a trihydroxy ethylated derivative of rutin, a naturally occurring bioflavonoid that is frequently present in grains of cereal, tea, coffee, variety of fruits and vegetables [11,12]. In several pathological circumstances, TROX has been demonstrated to prevent inflammation-induced tissue damage [13] and to successfully alleviate diabetes-related symptoms such as obesity, hyperlipidemia and insulin resistance [14-16]. Along with preventing the oxidative damage triggered by d-galactose and improving insulin signaling in vivo, TROX has been shown to have neuroprotective properties [17]. However, more study is required to completely understand the protective effects of TROX versus the development of T2DM, which is triggered by inflammation. The goal of this research is to ascertain how the positive impact of TROX on the brain might mitigate the negative effects of T2DM on cognitive performance.

METHODS

Mice

BALB/ c male mice at four weeks of age, were kept in an animal house with predetermined conditions (25 ± 1 °C temp, 55 ± 10 % relative humidity, 12 hr. light and 12 hr. dark phase) and allowed to acclimatize for two weeks. The animals in the experiment had unrestricted access to food and water. With approval from International Islamic University Islamabad's Ethical Committee (permission No. IIUI- SA- CIRBS/ FoS- EC 2022/3), the National Institute of Health's criteria were followed.

Experimental Groups, Drug Administration and Establishment of the T2DM Model in Mice

This study utilized 50 mice, with 10 mice assigned to each of the five experimental groups. Following the evaluation of metabolic data and behavioral tests, the mice in each group were randomly assigned to two equal subgroups. From each group, five mice (n = 5) were utilized for biochemical investigations, while the remaining five mice (n = 5) were assigned to histology analyses. The current article does not include the histology data. The standard food [18] was given to control and drug control (TROX) groups. The HFD group is concurrently adhered to the 60 % HFD. For 19 weeks, HFD+STZ mice were maintained on 60 % HFD diet, in order to induce diabetes, between week 12 and 15, they received five intraperitoneal injections of STZ at a dose of 60 mg/kg. From weeks 15 to 19, the control, drug control, HFD and HFD+STZ groups received intraperitoneal injections of 0.9% saline (0.5 ml/ Kg), whereas the HFD+STZ+TROX group received daily intraperitoneal injections of TROX (150 mg/Kg).

A 60 mg/kg of freshly made STZ were diluted in citrate buffer (pH 4.5) and was administrated intraperitoneally five times between weeks 12 to 15 following an overnight fast to create the T2DM model. In week 15, fasting blood glucose (FBG) level were measured using the On-Call Extra glucometer (Acon Laboratories Inc. San Diego, USA). The findings showed that the FBG level was higher than 200 mg/dl or 11.1 mmol/L, verifying a successful development of T2DM model.

Consumption of Food, Body Mass Index (BMI) and Body Weight

To ensure precise measurement and create a continuous consumption profile, the daily food intake of T2DM mouse model tracked for 19 weeks while food leftovers were left in the bedding. A weekly body weight and BMI record was kept in an effort to track changes. By dividing the body mass by the square of the length, the BMI was measured on a regular basis.

Blood glucose and Fasting Blood glucose (FBG)

Once a week the blood sample from the mice tail were taken and used to measure the blood glucose level on On- Call Extra glucometer. To improve accuracy the acquired data were averaged. In week 19, the blood glucose level was taken following an overnight fast, and the FBG was computed.

Glucose and Insulin Tolerance Assessment

For the assessment of glucose tolerance, the mice were given an intraperitoneal injection of a 20 % solution of glucose (2 mg/ g of body weight) after a six- hour fast. Mice received intraperitoneal injections of human insulin (0.25 IU/kg) after the same duration of fasting to determine their insulin tolerance. Treatment with 1 g/kg was implemented if the glucose level reached to 20 mg/dl or below. Blood was extracted from the tail vein at 0, 15, 30, 60, 90 and 120 minutes. The level of glucose in the blood was measured using a glucometer.

Behavioral Analysis

Before conducting behavioral analysis test, the mice were housed in habituation room that was kept at 25 ± 2 °C for 30 minutes. The Open Field Test, Elevated Zero Maze, Shallow Water Maze and Y-maze and were carried out according to previously established protocols [19-21]. For additional behavioral study, the activity in each trail was recorded on video.

Animal Dissection and Tissue Homogenate

To minimize discomfort, mice were profoundly anaesthetized with diethyl ether and placed to sleep in a small enclosure [22-24]. While the remaining animals were given intracardial perfusion with phosphate buffer saline at pH 7.4 for biochemical tests.

Reactive Oxygen Species Quantification

The ROS level was measured as described previously [25]. Fluorescence at excitation wavelengths of 484 nm and emission wavelength of 530 nm was measured using a Varioscan LUX 96-well plate reader. Without homogenate, the blank sample was incubated to account for DCFH-DA conversion and baseline fluorescence.

Determination of Lipid Peroxidation, Antioxidant activities and Nitric Oxide (NO) Concentration

Standard procedures were employed to quantify malondialdehyde (MDA) in the LPO study [26], catalase activity, GSH activity, SOD and NO was measured according to previously available literature [27].

Statistical Analysis

Using GraphPad Prism 8.0, parametric two-way and one-way ANOVA were followed by Tukey’s post hoc test for statistical analysis at a significance level of *P* < .05. Values less than .0001 were represented by ****, #### and $$$$. Also values fewer than .001 was denoted by ***, ### and $$$. Additionally, the standardized representation of the *p*-value that was employed was **, ## and $$ for values less than .01 and *, # and $ for values less than .05.

Group- wise comparisons were indicated using several statistical notations: * indicates comparisons between the control group and other groups, # indicates comparisons between the HFD group and other groups and $ indicates comparisons between the HFD+STZ group and HFD+STZ+TROX groups.

RESULTS

Troxerutin impact on Food Intake, Body Weight, BMI, and Adipose Tissue Weight

Food intake showed a significant variation among the experimental groups (F = 2029, *P* < .0001). The HFD group exhibited a markedly higher food consumption (60.39 ± 4.008 g) compared to both the control (45.88 ± 1.950 g) and drug control (46.29 ± 1.872 g) groups. The HFD+STZ group consumed significantly less food than the HFD group, yet their intake remained higher than that of control and drug control groups. Conversely, the HFD+STZ+TROX (treated) group demonstrated a significant decrease in food consumption relative to the HFD and HFD+STZ groups, while still maintaining higher intake levels than the control and drug control groups (Figure 1 A).

Body weight and weight gain were significantly higher in the HFD group versus the control and drug control groups. Compared to HFD, the HFD+STZ mice body weight and weight increase were noticeably lower. The HFD+STZ+TROX group gained less weight than the HFD group, but their body weight was noticeably greater in contrast of the drug control, HFD+STZ and control groups (Figure 1 B and D).

Significant disparities between groups were found via BMI analysis. The BMI of the HFD group was noticeable greater versus the drug control and control groups. HFD+STZ group had considerably lower BMI than the HFD group. HFD+STZ+TROX group had significantly higher BMI than the control, drug control and HFD+STZ groups but lower BMI versus the HFD group (Figure 1 C).

Adipose tissue weight varied significantly across groups. Contrary to the HFD+STZ and control groups, the HFD group adipose tissue weight was noticeably greater. Adipose tissue mass was substantially lower in the HFD+STZ+TROX group than the HFD+STZ, although it was substantially higher in the HFD+STZ group (Figure 1 E).

Troxerutin Reduces Hyperglycemia in T2DM Mouse Model

Blood glucose levels varied significantly across groups (F=284.0, p<0.0001). In contrast to the control and drug control groups the HFD group blood glucose level was noticeably higher. When contrasted with all other groups, the HFD+STZ group had most elevated blood glucose levels. Blood glucose level in the HFD+STZ+TROX group were lesser than those seen in the HFD and HFD+STZ groups, while exceeded from the control and drug control groups (Figure 2 A).

Glucose tolerance test showed significant differences (F=333.7, p<0.0001). The glucose tolerance of the HFD and HFD+STZ groups was noticeably lower than the drug control and control groups. The HFD+STZ+TROX group showed improved glucose tolerance versus the HFD and HFD+STZ groups (Figure 2 B).

Insulin tolerance test revealed significant differences (F=2927, p<0.0001). Following insulin administration, the HFD and HFD+STZ groups blood glucose levels were noticeably superior versus the control and drug control groups. Elevated blood glucose levels were noted in the HFD+STZ+TROX group versus the drug control and control groups, but lower than HFD and HFD+STZ groups (Figure 2 C).

Fasting blood glucose levels also varied significantly (F=29.93, p<0.0001). Significantly higher FBG levels were noted in the HFD and HFD+STZ groups versus the control and drug control groups. FBG levels in the HFD+STZ+TROX group were lower than the ones in the HFD+STZ group, but greater versus the drug control and control groups (Figure 2 D).

Effect of TROX on Anxiety-like Behaviour

HFD+STZ mice showed significantly reduced peripheral crossing frequency versus the control group. Peripheral crossing frequency was higher in the HFD+STZ+TROX mice than in HFD and HFD+STZ groups, but there was no discernible difference from the control group (Figure 3 A). The frequency of central crossings was higher in HFD and HFD+STZ mice versus control group. The central crossing frequency was considerably lower in HFD+STZ+TROX mice versus HFD+STZ and HFD groups (Figure 3 B). In contrast to the control group, HFD and HFD+STZ spent substantially more time in the periphery. While the HFD+STZ+TROX group spent noticeably less time in the periphery against the HFD+STZ group (Figure 3 C). HFD and HFD+STZ mice spent significantly fewer time in the center versus the control group. HFD+STZ+TROX mice consumed significantly more time in the center compared to the HFD+STZ and HFD groups (Figure 3 D). In comparison to the control group, the number of rearing's were lower in HFD and HFD+STZ mice. The rearing frequency of HFD+STZ+TROX mice was higher in contrast to that of the HFD+STZ group, but there was no discernible difference between the HFD and control groups (Figure 3 E).

TROX's Effect on Behavior in the Elevated Zero Maze Test

The amount of head dips was considerably lower in the HFD+STZ and HFD groups versus the control group. However, the HFD+STZ and HFD groups didn't vary significantly. It's notable to note that the HFD+STZ+TROX group significantly increased versus to the HFD and HFD+STZ groups, bud didn't vary from the control group (Figure 4 A). HFD+STZ mice didn't significantly vary from the HFD group, however they did enter the open quadrant less frequently than the control group. The HFD mice also showed a notable difference from the control group. The HFD+STZ+TROX shown a considerable increase in comparison to the HFD and HFD+STZ groups, bud it didn't vary from the control group (Figure 4 B).

In contrast to control group, HFD+STZ mice visited the close quadrant more frequently, but they did not vary substantially from the HFD group. There were also notable differences between the HFD and the control groups. The HFD+STZ+TROX had a considerable decline in comparison to the HFD+STZ group, but did not vary considerably from the control and HFD groups (Figure 4 C). Although the HFD+STZ group didn't differ substantially from the HFD group, they did have a much larger number of SAPs than the control group. There were also notable differences between the HFD and the control groups. There were no discernible variations in the number of SAPs between the control, HFD and HFD+STZ+TROX groups. In contrast to the HFD+STZ, the HFD+STZ+TROX group demonstrate a notable decline (Figure 4 D).

The HFD+STZ and HFD groups didn't vary substantially from one another, although they didn't spend a longer period in the open quadrant than the control group. While the HFD+STZ+TROX group did spend considerably longer periods of time in the open quadrant than the HFD and HFD+STZ groups (Figure 4 E). In contrast to the HFD and HFD+STZ groups, the control group spent shorter periods in the closed quadrant. There was no discernible difference between the HFD and HFD+STZ groups. Although the HFD+STZ+TROX group did not vary significantly from the control group, it spent less time in the closed quadrant and shown significant improvements compared to the HFD and HFD+STZ groups (Figure 4 F).

The Effect of TROX Therapy on T2DM Mice Learning Memory and Spontaneous Cognitive Memory

The Shallow Water Maze (SWM) was used to assess memory and learning functions. The HFD+STZ group took considerably longer to escape versus the control group, and didn't vary significantly from the HFD group. Also, the HFD group took substantially longer than the control group. The HFD+STZ+TROX group escaped notably faster against the HFD and HFD+STZ groups but did not differ significantly from the control mice (Figure 5 A). The HFD+STZ mice made notably more errors versus the control mice, and didn't vary considerably from the HFD mice. The HFD mice also crafted significantly more errors against the control mice. The HFD+STZ+TROX group made fewer errors than the HFD and HFD+STZ mice but didn't fluctuate significantly from the control mice (Figure 5 B).

The HFD+STZ mice consumed a longer period in the SWM versus the control mice, and didn't contrast with the HFD group. The HFD group also spent more time than the control mice. The HFD+STZ+TROX mice spent less time than the HFD and HFD+STZ mice but didn't fluctuate notably from the control mice (Figure 5 C). In Y-maze, the HFD+STZ and HFD groups entered the novel arm less frequently than the control mice, but didn't vary substantially from one another. The HFD+STZ+TROX mice entered into novel arm more repeatedly than the HFD and HFD+STZ groups but didn't contrast significantly from the control mice (Figure 5 D). The overall number of arm entries for each group didn't differ significantly (Figure 5 E).

The HFD+STZ and HFD groups consumed a shorter period in the novel arm versus the control mice, but didn't vary considerably from each other. The HFD+STZ+TROX mice consumed a longer period in the novel arm against the HFD and HFD+STZ mice but didn't fluctuate significantly from the control mice (Figure 5 F). The control group revealed higher spontaneous alternation compared to HFD and HFD+STZ mice, which didn't differ substantially from each other. The HFD+STZ+TROX group showed higher spontaneous alternation compared to HFD and HFD+STZ mice but didn't vary considerably from the control mice (Figure 5 G).

TROX Positively Regulates ROS, LPO, Antioxidant efficiency, and NO Production

TROX therapy significantly impacted oxidative stress markers in the brain. The ROS levels in the HFD+STZ and HFD mice showed noteworthy increase against the control group. HFD+STZ+TROX group exhibited significantly lower ROS levels versus the HFD and HFD+STZ mice (Figure 6 A). LPO levels were substantially elevated in HFD+STZ and HFD mice versus the control mice. HFD+STZ+TROX mice revealed notably inferior LPO levels compared to HFD and HFD+STZ mice (Figure 6 B).

Catalase activity was significantly decreased in HFD and HFD+STZ groups versus the control mice. HFD+STZ+TROX group showed significantly increase catalase activity against the HFD and HFD+STZ groups (Figure 6 C). GSH activity was significantly decreased in HFD and HFD+STZ groups versus the control mice. HFD+STZ+TROX group showed substantially enhanced GSH activity against the HFD and HFD+STZ groups (Figure 6 D).

SOD activity was considerably lowered in HFD and HFD+STZ mice compared to the control mice. HFD+STZ+TROX mice showed notable improved SOD activity against the HFD+STZ group (Figure 6 E). NO levels in both blood serum and brain tissue were significantly elevated in HFD+STZ and HFD mice, compared with the control mice. HFD+STZ+TROX group showed significantly decreased NO levels against the HFD and HFD+STZ mice (Figure 6 F and G).

Figures

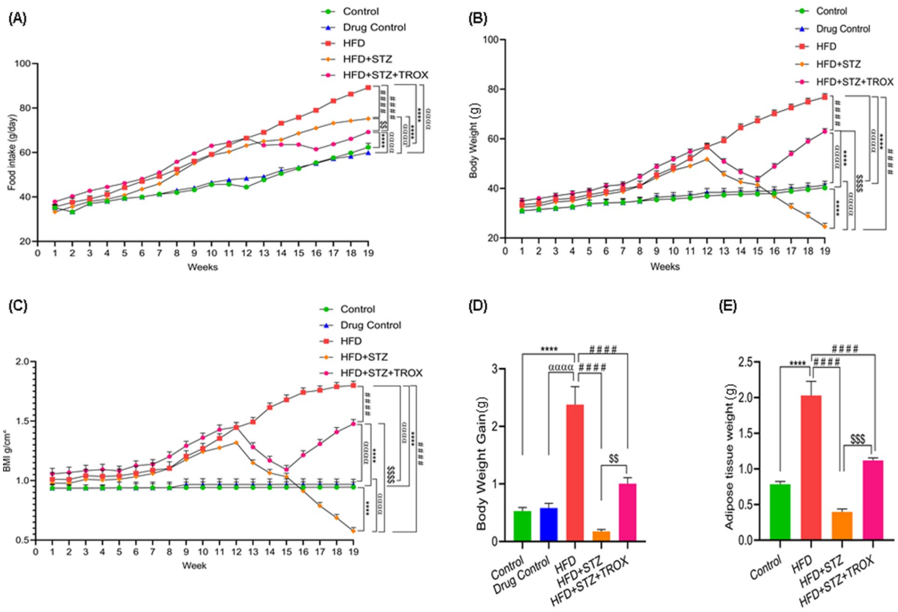

Figure 1: Demonstrated an effect of TROX on daily food consumption, weekly body weight, body weight gain, BMI and on adipose tissue weight. Panel (A) showed a comparison of the daily food intake. Panel (B) indicated weekly variations in body weight. Panel (C) displayed a shift in BMI. Panel (D) showed a body weight

increase profiles, while the panel (E) disclosed the weight of adipose tissue in different groups. The data (n = 10) is displayed as mean ± SEM.

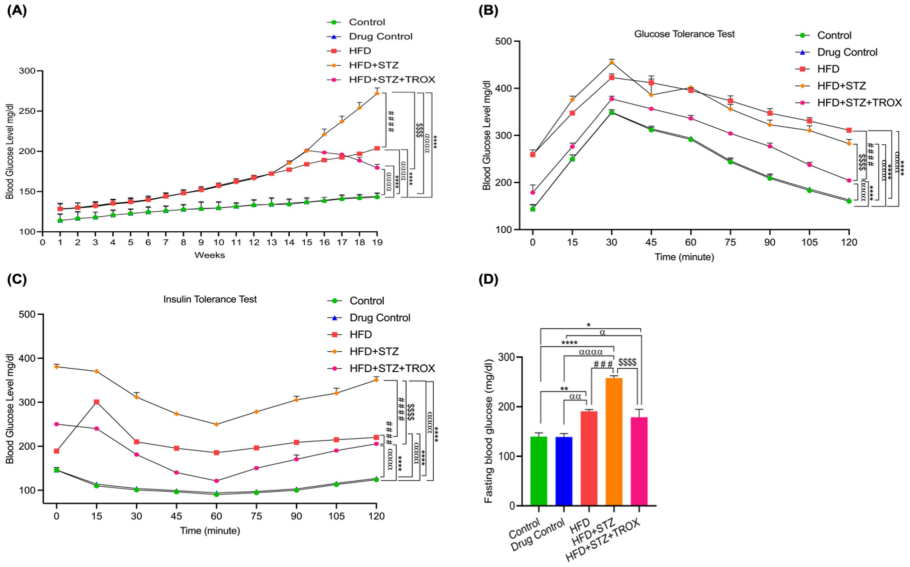

Figure 2: Troxerutin effect on hyperglycemia in HFD mouse model. Panel (A) shown a weekly blood glucose fluctuation. Panel (B) illustrated a glucose tolerance testing. Panel (C) showed an insulin tolerance testing and a panel (D) exhibited a summary of fasting blood glucose readings. The data shown here is the mean ± SEM.

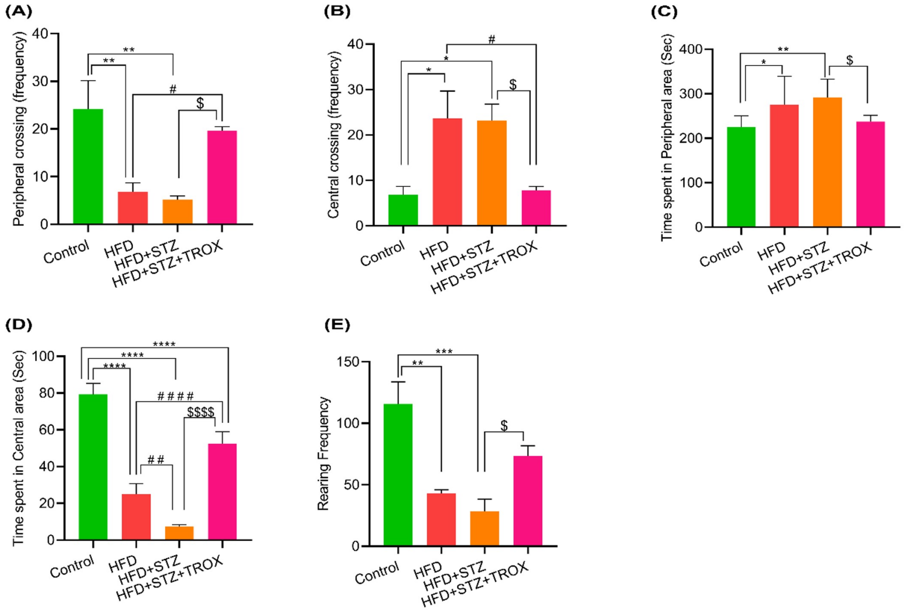

Figure 3: The following metrics were used to evaluate the outcome of TROX therapy on anxiety-like behavior: (A) peripheral crossing frequency, (B) center crossing frequency, (C) peripheral area time, (D) central area time and (E) rearing frequency. The reported data is the mean ± SEM for a sample size of ten (n = 10).

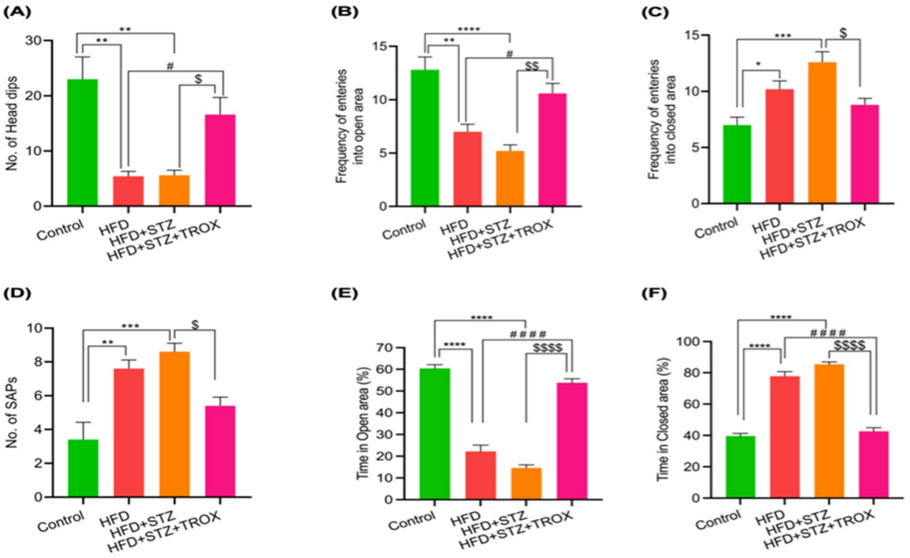

Figure 4: Effects of TROX therapy on behavioral presentation in the elevated zero maze test. Panel (A) represents the Head dips frequency. Panel (B) demonstrate the number of open quadrant entries while the panel (C) displayed the number of entries into closed quadrant. Panel (D) shown the stretch-attended postures (SAPs) alterations. Panel (E) show how long mice stay in the open quadrant, while the panel (F) demonstrate the amount of time mice spent in the closed quadrant. The sample size of ten (n = 10) is represented by mean ± SEM.

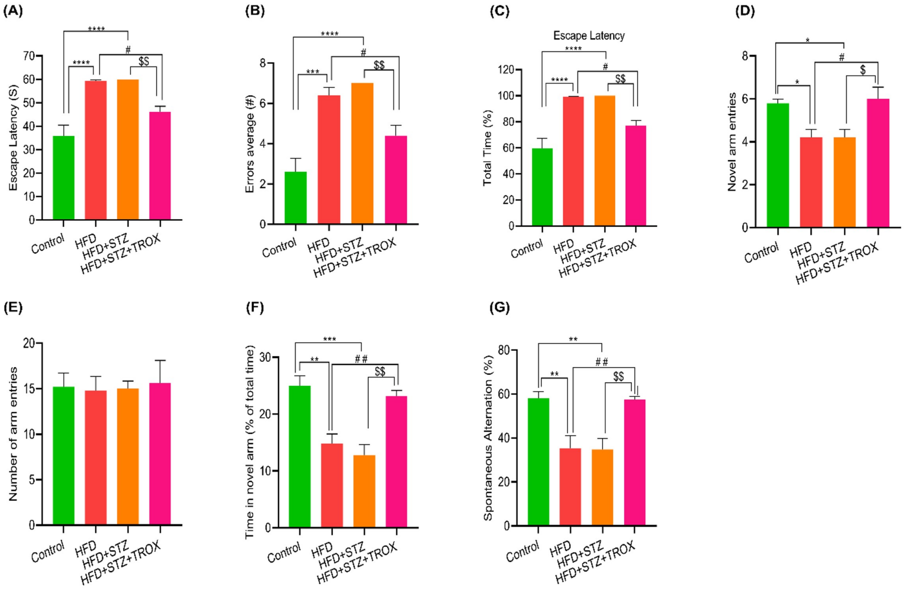

Figure 5: Variations in group behavior of Y-Maze and SWM test. The panel (A) showed escape latency, (B) the number of errors, (C) total time spent in escape latency (%). Panel (D) indicated how many new arm entries there were in the Y maze, (E) the amount of arm entries in total, (F) total amount of time consumed in the new arm (%) and (G) percentage of spontaneous alternation. The data displayed is mean ± SEM.

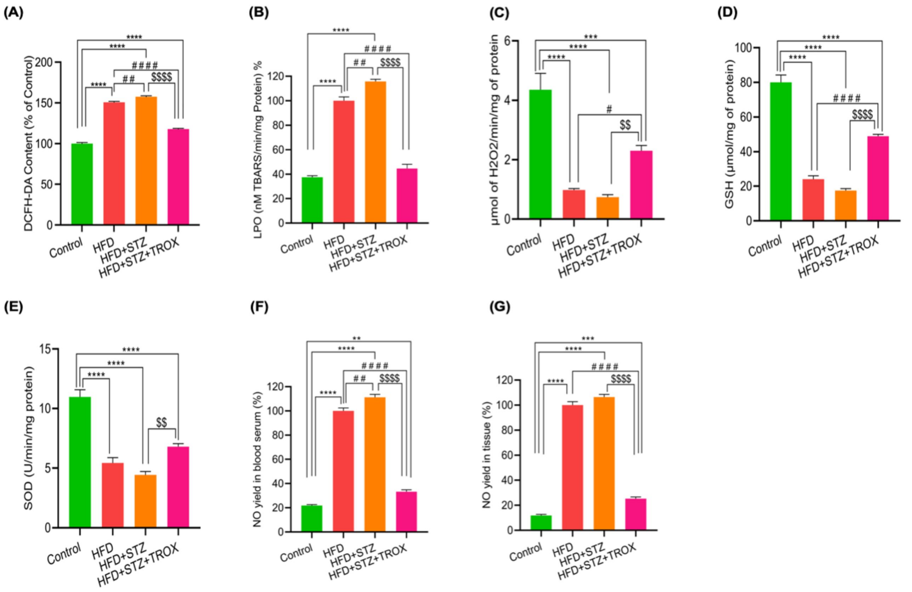

Figure 6: TROX therapy effect on ROS, LPO, antioxidant enzymes and NO in T2DM model of mice. Panel (A) shows the dynamic ROS generation. (B) emphasizes dynamic variation in LPO levels. (C) indicate the dynamic fluctuation of catalase activity. (D) Reveals variations in GSH activity. (E) displays variations in SOD activity. (F) presents variation in NO activity in blood serum and (G) shows the NO activity in brain tissue. With a sample size of five (n = 5), the data given is mean ± SEM.

DISCUSSION

T2DM is a serious metabolic disorder, indicated by excessive blood glucose level and can damage several organs, including the brain [28]. It is closely linked to neuroinflammation, which causes neuronal cell death and higher risk of cognitive impairment, which in turn raises the risk of dementia [29]. TROX administration has been revealed to significantly downgrade the blood glucose levels in mice given a high cholesterol and a high fructose diet. It also lower levels of reactive oxygen species and advance glycation end products [30]. In this study, we demonstrated that TROX considerably improve the cognitive impairment caused by T2DM. This study found that TROX reduced anxiety, body weight, food intake, glucose level and enhances cognitive function in mice with learning and memory deficits brought on the T2DM. The results of earlier research were utilized to calculate the dose of TROX [16,30,31].

In order to examine their diverse and significant effects on metabolic parameters and the development of T2DM, we compared high-fat, T2DM and suitable normal control meals in this study. The T2DM model was created by repeatedly giving a modest dose of STZ (60 mg/ kg) intraperitoneally together with HFD, which is persistent with the findings of earlier research [32,33].

Compared to the control mice, the HFD+STZ mice indicated a substantial decline in body weight from week 15 th to 19 th, indicating the induction of T2DM. Increased food consumption, a decrease in body weight, BMI and adipose tissue weight are all associated with this induction of T2DM. Additionally, there was a substantial rise in weekly glucose levels and also higher FBG level in the HFD+STZ mice. Also, in testing of glucose and insulin tolerance, the HFD+STZ group showed less resistance. This animal model's mechanism for decrease body weight and hyperglycemia is a biomarker for animal health and is linked to motor dysfunction, cognitive decline and T2DM related acute phase dementia [33-35]. The Trox caused a significant decrease in food consumption and reversed the pathological weight loss as observed in case of untreated diabetic group. Additionally, TROX treatment promotes the accumulation of adipose tissue, which improves body weight growth and BMI. Additionally, TROX therapy significantly raised insulin and glucose tolerance while lowering weekly glucose and fasting blood glucose levels. According to similar findings, TROX may help in managing diabetes, enhance general metabolic and degenerative health and assist diabetic patients in managing their condition [36,37].

Furthermore, in this research, we sought to demonstrate a link between metabolic abnormalities and anxiety, as well as memory dysfunction in the T2DM model. We utilized the typical behavioral assessments such as open field, elevated zero maze test, SWM, and Y-Maze tests to calculate dysfunction of cognition. Our behavioral investigation illustrated that, compared to the HFD+STZ group in open field test the TROX therapy significantly increased total rearing, peripheral crossing, and the amount of time consumed in both central region. TROX therapy also shows a significant decrease in central crossings and in the time spent in the peripheral area of open field test. As contrasting to TROX treatment, the HFD+STZ mice consumed a lot of time in the periphery region. Throughout the elevated zero maze test and in the open field test the raised anxiety levels or actions of HFD+STZ group were more noticeable, when paralleled with the HFD+STZ+TROX group. The patterns of enhanced behavior in the SWM and Y-maze test outcomes were comparable. These outcomes align with earlier findings showing the neuroprotective properties of TROX in a variety of settings, such as oxidative stress, neuroinflammation, memory loss, and diabetes type 1 mouse models [19,37,38]. In the final analysis, our outcomes on ROS are consistent with prior studies showing that therapy with TROX successfully reduced ROS levels, as demonstrated in a rat model of diabetic cardiomyopathy for T2DM [31] and in biological and therapeutic action of TROX [36]. The current study revealed a considerable disruption in the antioxidant enzyme concentrations in the HFD+STZ mice, as evidenced by a considerable rise in LPO and a significant drop in SOD, GSH and catalase. However, therapy with TROX indicated a considerable enhancement in the control of antioxidant enzyme levels. These findings are constant with other investigations that highlighted the impact of TROX on antioxidant enzymes [30,39,40]. Our hypothesis that TROX possesses potent anti-apoptotic, neuroprotective, anti-inflammatory and antioxidant properties is supported by our data. TROX has been found to be neuroprotective in a number of different neurological illness models [41,42].

In summary, the current research highlights the possible potential of TROX as a novel candidate capable of counteracting the cognitive impairment and metabolic parameters associated with T2DM. Beside the physiological markers such as altered glucose level and body weight, TROX due to its strong antioxidant nature appears to have a neuroprotective role. Our findings also suggest that TROX not only mitigate the oxidative stress but also plays a key role in cognitive improvement, setting it an ideal candidate for preventing T2DM. However, further research in relevant chronic and clinical models of T2DM is required to explore the full mechanism of action related to human health.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Waqas Ahmad contributed to the conceptualization, methodology, experimentation, data analysis, and writing of the original draft. Ashfaq Ahmed Khan Malik was responsible for assisting in methodology development and experimental execution. Farhan Younas played a key role in supervision and finalization of the manuscript. Muhammad Raiz contributed to the validation and interpretation of the data. Ikram Ullah was involved in experimental design, project administration, supervision, and critical review and editing of the manuscript.

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