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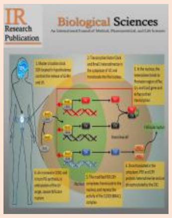

Eriodictyol Ameliorates Biochemical and Histopathological Abnormalities in the Hippocampal Brain Region of Fructose/Streptozotocin-Induced Type-2 Diabetic Rats

Anthony Monday Agesin[1], Afolabi Clement Akinmoladun[2]

[1]Department of Science Laboratory Technology, Biochemistry Unit, Faculty of Applied Sciences, Rufus Giwa Polytechnic Owo, P.M.B 1019, Nigeria.
[2]Department of Biochemistry, School of Sciences, The Federal University of Technology, P.M.B. 704, Akure, Nigeria.

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ABSTRACT

Eriodictyol offers essential protection to the brain but its effect in diabetes-induced neurologic dysfunction is poorly known. Diabetes was induced in male Wistar rats by administering 10% fructose in the drinking water for fourteen days followed by single intraperitoneal injection of 40 mg/kg body weight streptozotocin. Separate groups of diabetic rats were post-treated with varying doses of eriodictyol (0.25, 0.5 and 1.0 mg/kg) for 14 days. Thereafter, the animals were sacrificed; the hippocampi were carefully separated and processed for biochemical estimations and histopathological examination. The weight, blood glucose level, and glycated hemoglobin were determined using standard methods. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Acetylcholinesterase (AChE) and Na^+/K^+-ATPase activities were evaluated. Reduced glutathione (GSH), protein carbonyl (PC) levels and the extent of lipid peroxidation (LPO) were also evaluated. Also, histopathological examination was carried out on the hippocampi brain region. Eriodictyol significantly ($p<0.0001$) reduced the high blood glucose level and glycated hemoglobin in the diabetic rats. There was a significant ($p<0.0001$) increase in SOD, CAT, GPx, Na^+/K^+-ATPase activities and weight as well as GSH level but, decrease in AChE activity and extent of LPO, and PC levels of diabetic rats post-treated with the flavonoid. These results were consistent with the histopathological findings which showed significant attenuation of neuronal cell death in diabetic rats post-treated with the flavonoid. The data established for the first time, the ameliorative potential of eriodictyol on oxidative imbalance and histopathological changes against fructose/stz-induced type-2 diabetic rats.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by prolonged high blood sugar levels, resulting from either the destruction of insulin-producing pancreatic cells or impaired insulin action in target tissues [1]. The prevalence of DM, particularly type-2 diabetes (T2D), is increasing

**Address for correspondence*
Department of Science Laboratory Technology, Biochemistry Unit, Faculty of Applied Sciences, Rufus Giwa Polytechnic Owo, P.M.B 1019, Nigeria.
Email: agesinanthony_Monday@rugipo.edu.ng

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at an alarming rate and could reach epidemic proportions if research does not focus more on prevention than on curative strategies [2]. This rise in T2D is further complicated by the complexity and wide range of associated complications [3].

The brain, which serves as the central organ of the nervous system in vertebrates and most invertebrates, is an insulin-sensitive organ with insulin receptors expressed throughout its distinct regions [4]. The brain relies on approximately 25% of the body's total glucose for proper function [5]. A constant supply of glucose is critical for maintaining normal brain metabolism, neuronal vitality, signal transmission, neurotransmission, synaptic plasticity, and cognitive function. However, severe fluctuations in glucose levels can disrupt these vital processes [5, 6].

The brain contains approximately 55–70 billion neurons connected by synapses, allowing communication via long protoplasmic fibers called axons [7]. These axons transmit action potentials, which are signals carried to distant parts of the brain or body, targeting specific recipient cells [7]. The brain’s primary function is to exert centralized control over other organs in the body. Among the brain’s distinct regions is the hippocampus, which is small, curved structure located deep within the brain’s temporal lobes, one on each side of the brain [8]. It is a part of the limbic system, which plays a crucial role in regulating emotions, memory, and spatial navigation. The hippocampus is particularly important for the formation, organization, and storage of new memories, especially long-term memories [8, 9]. It is also involved in consolidating short-term memories into long-term ones and helps with spatial memory, which allows for navigation and understanding of one’s environment [9]. Damage to the hippocampi can result in memory problems and disorientation, as seen in conditions like Alzheimer's disease and other forms of dementia [10]. The hippocampus is also sensitive to stress and plays a role in the regulation of mood [10].

Neurologic dysfunction refers to disorders of the central nervous system that impair the brain’s ability to process efficiently. When this dysfunction becomes severe, the brain cannot compensate, leading to detrimental effects on learning, development, and rehabilitation, ultimately affecting daily functions [11].

Flavonoids, also known as bioflavonoids, are the most abundant polyphenolic compounds in the human diet and are secondary metabolites found in plants and fungi. Structurally, they consist of a 15-carbon skeleton with two phenyl rings and a heterocyclic ring [12]. Their chemical properties vary based on structural class, degree of hydroxylation, and polymerization. Flavonoids have garnered attention for their health benefits, particularly their antioxidant activity, ability to induce human enzymes, and potential to prevent radical generation that damages key biomolecules [13]. Dietary therapies rich in flavonoids offer promising preventive and management strategies for T2D, as epidemiological studies have shown an inverse relationship between polyphenol-rich diets and the risk of chronic diseases like T2D [12, 13]. Flavonoids have also been found to protect against various infectious and degenerative diseases [14]. Eriodictyol, a flavonoid-rich compound, acts as an antioxidant that modulates oxidative stress in the body [14]. Free radicals, byproducts of human cell metabolism, contribute to life-threatening conditions such as coronary heart disease, obesity, T2D, and cancer [15]. Flavonoids help neutralize the harmful effects of reactive oxygen and nitrogen species, offering protection against these diseases [15].

Materials and Methods

Chemicals and Reagents

Eriodictyol and streptozotocin (STZ) were obtained from Santa-Cruz Biotechnology (Darmstadt, Germany). Reduced glutathione (GSH), adenosine triphosphate (ATP), 5',5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman’s reagent), epinephrine, 2,4-dinitrophenyl hydrazine (DNPH), 6,7-Dimethyl-5,6,7,8-Tetrahydropterine (DMTHP), hydrogen peroxide, fructose, and potassium chloride were purchased from Sigma-Aldrich (St-Louis, MO, USA). Total protein assay kits were sourced from Randox Laboratories (Antrim, U.K.). All other chemicals and reagents used were of analytical grade.

Experimental Animals and Induction of T2DM using the Fructose/STZ Model

Fifty (50) adult male Wistar rats, weighing between 200-230 g, were procured from the Department of Veterinary Anatomy, University of Ibadan. The animals were acclimatized for two weeks, fed standard rat chow (Vital feed), and provided with water ad libitum in the Animal House of the Department of Biochemistry, The Federal University of Technology, Akure, Nigeria. Handling and usage of the animals followed the NIH Guide for the Care and Use of Laboratory Animals (2011), and the study was approved by the institutional Committee for the Ethical Use of Research Animals at the Federal University of Technology, Akure.

The animals were divided into five groups of ten animals each. Type-2 diabetes was induced based on the method of Obafemi et al. [16]. Except for the negative control group, all animals received 10% fructose for 14 days. On the 15th day, after overnight fasting, streptozotocin (STZ) (40 mg/kg body weight) in ice-cold 0.1 M citrate buffer (pH 4.5) was administered intraperitoneally. Blood glucose levels were checked 72 hours post-STZ administration using an Accu-check® glucometer, and animals with glucose levels ≥ 250 mg/dl were considered diabetic. The groups were as follows:

Group 1: Control

Group 2: Diabetic

Group 3: Diabetic + 0.25 mg/kg Eriodictyol

Group 4: Diabetic + 0.5 mg/kg Eriodictyol

Group 5: Diabetic + 1 mg/kg Eriodictyol

Eriodictyol doses were determined based on literature reports [17]. Treatments lasted 21 days, and 24 hours after the final dose, animals were sacrificed. Blood samples were collected by cardiac puncture, and serum was used for glycated hemoglobin estimation.

Biochemical Estimations

The hippocampi were dissected in ice-cold 1.15% potassium chloride solution and blotted dry. The tissues were homogenized in 10% phosphate-buffered saline (PBS, pH 7.4) (1:10 w/v) using a Teflon homogenizer, followed by centrifugation at 10,000 x g at 4°C for 30 minutes. The supernatant was collected for biochemical analyses, and histopathological evaluation was performed on the hippocampi brain regions.

Estimation of brain total protein

Total protein concentration was measured using the Biuret method with a Randox assay kit. Brain homogenate (0.1 mL) was diluted with 3.9 mL of 0.9% saline to create a 1 in 40 dilution. Three milliliters of Biuret reagent was added to 2 mL of the diluted sample, and the mixture was incubated at room temperature for 30 minutes before absorbance was read at 546 nm.

Assessment of diabetic markers

Estimation of glycated hemoglobin

Glycated hemoglobin was measured following the method of John et al. [18]. Venous blood was collected in an EDTA-containing tube and centrifuged. Packed red cells were hemolyzed in 2 mL distilled water on a vortex mixer. The hemoglobin fractions were separated by selective adsorption using a boronate-containing insoluble matrix, with unmodified hemoglobin being washed through and glycosylated hemoglobin eluted for measurement at 414 nm.

Determination of blood glucose level

Fasting blood sugar (FBG) levels were determined using an Accu-Check glucometer [16]. A tail snip was performed, and blood was applied to the glucometer strip. The value was read from the glucometer screen.

Oxidative stress markers

Determination of reduced glutathione (GSH) level

The level of GSH was estimated according to the method of Beutler [19]. Brain homogenate (0.2 mL) was added to 1.8 ml of distilled water and 3 mL of 4 % sulfosalicyclic acid (SSA) was mixed with the brain homogenate. The mixture was then allowed to stand for approximately 5 min and then filtered. One mililitre of filtrate was added to 4 mL of 0.1 M phosphate buffer (pH 7.4). Finally, 0.5 mL of the Ellman's reagent was added. A blank was prepared with 4 mL of 0.1 M phosphate buffer pH 7.4, 1 mL of diluted precipitating solution (3 parts to 2 parts of distilled water) and 0.5 mL of the Ellman's reagent. The absorbance was read at 412 nm against the reagent blank. GSH was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard (A stock solution of 0.1M GSH standard curve).

Evaluation of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was determined according to the method of Haque et al. [20].Five hundred microliters of 0.1 M phosphate buffer (pH 7.4), 100 µL of 10 mM sodium azide, 200 µL of 4 mM GSH, 100 µL of 2.5 mM H_2O_2 were added to 500 µL of the brain homogenate sample, after which 600 µL of distilled water was added and mixed thoroughly. The reaction mixture was incubated at 37°C for 3 min after which 0.5 mL 10% TCA was added and centrifuged at 3000 rpm for 5 min. To 1 mL of each of the supernatants, 2 mL of K2HPO4 and 1 mL of 0.04% DTNB were added and the absorbance was read at 412 nm against a blank (H_2O).

Evaluation of catalase activity

Catalase activity was determined according to the method of Pandey et al. (21). Brain homogenate (100 μL) was mixed with H_2O_2 (1000 μL) and then incubated at 37°C for 3 min, after that ammonium molybdate (32.4 mmol/L) was added. After that the tubes were kept at room temperature. Changes in absorbance were recorded at formation of chromic acetate. After cooling at room temperature, the absorbance was read at 374 nm. The hydrogen peroxide content of the withdrawn sample was determined by the method described above.

Table 1. Effect of eriodictyol post-treatment on blood glucose level in fructose/streptozotocin-induced diabetic rats.

	Glucose (mg/dL) Before treatment	Glucose (mg/dL) After treatment	Change in glucose level (%)
Control	119.64 ± 4.71	118.00 ± 4.32	< 1.39
Diabetic (40 mg/kg)	293.09 ± 10.16 ^{a}	341.29 ± 22.44 ^{a}	> 16.45
Diabetic + Eriodictyol (0.25 mg/kg)	303.00 ± 15.58	211.42 ± 14.15 ^{b}	< 21.97
Diabetic + Eriodictyol (0.5 mg/kg)	311.55 ± 16.67	202.57 ± 8.03 ^{b}	< 35.16
Diabetic + Eriodictyol (1 mg/kg)	294.72 ± 10.75	175.71 ± 11.88 ^{b}	< 37.66

Results are expressed as mean ± SD (n=10). Values with a are significantly (p<0.0001) different from the control while values with b are significantly (p<0.0001) different from the diabetic group. <: Decrease; >: Increase.

Evaluation of superoxide dismutase (SOD) activity

SOD activity in the brain homogenate was determined by the method of Kakkar et al. [22]. The brain homogenate (1 mL) of the various groups was diluted in 9 ml of distilled water to make a 1 in 10 dilutions. An aliquot of the diluted sample (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was initiated by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline and was quickly mixed by inversion. The reference cuvette contained 2.5 mL of buffer, 0.3 mL of substrate (adrenaline) and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

Table 2. Effect of eriodictyol post-treatment on body weight of fructose/streptozotocin-induced diabetic rats

	Body weight(g) Initial	Body weight (g) Final	% Change in body weight (g)
Control	224.69 ± 3.42	239.15 ± 6.08	+6.04 ± 2.72
Diabetic (40 mg/kg)	220.84 ± 4.20^{a}	192.19 ± 10.51^{a}	-12.93 ± 4.75^{a}
Diabetic + 0.25 mg/kg Erio	210.26 ± 5.31^{b}	226.40 ± 4.75^{b}	7.12 ± 3.48^{b}
Diabetic + 0.5 mg/kg Erio	208.65 ± 4.23 ^{b}	229.85 ± 6.40^{b}	9.22 ± 5.58^{b}
Diabetic + 1 mg/kg Erio	212.52 ± 6.77 ^{b}	293.67 ± 8.45^{b}	11.32 ± 3.68^{b}

Results are expressed as mean ± SD (n=10). Values with a are significantly (p<0.0001) different from the control while values with b are significantly (p<0.0001) different from the diabetic group. Erio: Eriodictyol; -: Decrease; +: Increase.

Evaluation of extent of lipid peroxidation

Lipid peroxidation was evaluated by measuring the level of malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), according to the method described by Varshney and Kale [23]. Lipid peroxidation was calculated with a molar extinction coefficient of 1.56 3 105M21 cm21 and was expressed as units/mg protein.

Determination of protein carbonyl (PC) content

Protein carbonyl content in the brain regions was determined according to the method of Levine et al. [24]. Brain supernatant was incubated with DNPH for 60 min at room temperature. Following precipitation by adding 20% trichloroacetic acid, the pellet was washed with acetone and dissolved in 1 mL of Tris buffer containing sodium dodecyl sulphate (8% w/v, pH 7.4). The absorbance was measured at 360 nm and expressed as nmol carbonyls/mg protein.

Evaluation of acetylcholinesterase (AChE) activity

AChE activity was measured by the spectrophotometric method developed by Ellman et al. [25]. A volume 0.1 mL of 0.01 M DTNB was added to 2.6 mL of 0.1 M phosphate buffer (pH 8.0), 0.04 mL of brain homogenate was added to the above mixture followed by incubation for 5 min. After incubation, 0.04 mL of substrate (0.075 M acetylcholine iodide) was added to the reaction mixture. Absorbance readings were taken at 420 nm continuously for 3 min at 30 seconds intervals. The results were expressed in μmol-1min-1mg protein-1 using a molar extinction coefficient of 1.36 x 10-4M-1cm-1

Evaluation of Na^+/K^+-ATPase activity

Na^+/K^+-ATPase activity was assayed according to Svoboda and Mosinger [26]. Na^+/K^+-ATPase activity was assayed by taking 25 μL of Tris-HCl buffer (40 mM, pH 7.5) followed by the addition of 50 μL of 600 mM NaCl, 50 μL of 50 mM KCl, along with 50 μL of 1 mM Na-EDTAand 50 μL of 80 mM ATP. The reaction mixture was pre-incubated at 37°C for 10 min. Then, 25 µL of homogenate was added to the reaction mixture and further incubated at 37°C for 1 hour. The reaction was immediately arrested by the addition of 500 µL of 10% TCA. The precipitate was removed by centrifugation at 3,500 rpm for 10 min. To 50 µL of the supernatant, 925 µL of distilled water, 125 µL of ammonium molybdate and 50 µL of ANSA (7.4 mg/ml) 1-amino-2-naphtol-4-sulphonic acid) were added and incubated for 10 min at 37°C. The intensity of blue colour was read at 640 nm using spectrophotometer against a blank that contained all the reagents without the supernatant. Results were expressed in nanomoles of inorganic phosphorus liberated/min/mg protein.

Histopathological Evaluation

Histopathological assessment was carried out according to Day et al. [27].

Data Analysis

Data were analyzed using analysis of variance (ANOVA), followed by Fisher's LSD multiple comparison tests. Where appropriate, Duncan's multiple range test was also applied. Statistical significance was set at $P < 0.05$ for all tests. The data analysis was performed using GraphPad Prism 6.01 (GraphPad Software Inc., CA, USA).

Results and Discussion

The effects of eriodictyol on blood glucose levels in fructose/streptozotocin-induced diabetic rats are shown in Table 1. The diabetic group experienced a 16.45% rise in blood glucose, while there was a 1.39% decrease in the control group. In contrast, eriodictyol treatment at doses of 0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg led to blood glucose reductions of 21.97%, 35.16%, and 37.66%, respectively.

Additionally, the changes in body weight of diabetic animals post-treated with varying doses of eriodictyol are presented in Table 2. Diabetic animals exhibited a 12.66% decrease in body weight compared to a 6.93% increase in the control group. However, treatment with eriodictyol at doses of 0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg resulted in weight increase of 7.12%, 9.22%, and 11.32%, respectively.

Eriodictyol significantly ($p<0.0001$) reduced the high glycated hemoglobin in the blood of the diabetic rats and significantly ($p<0.0001$) increased the GSH level, SOD, CAT, GPx, Na^+/K^+-ATPase activities but significantly ($p<0.0001$) decreased the AChE activity, extent of LPO, and PC levels of diabetic rats post-treated with the various doses of the flavonoid as shown in figures; 1, 2, 3, 4, 5, 6, 7, 8 and 9 respectively when compared to the positive control group.

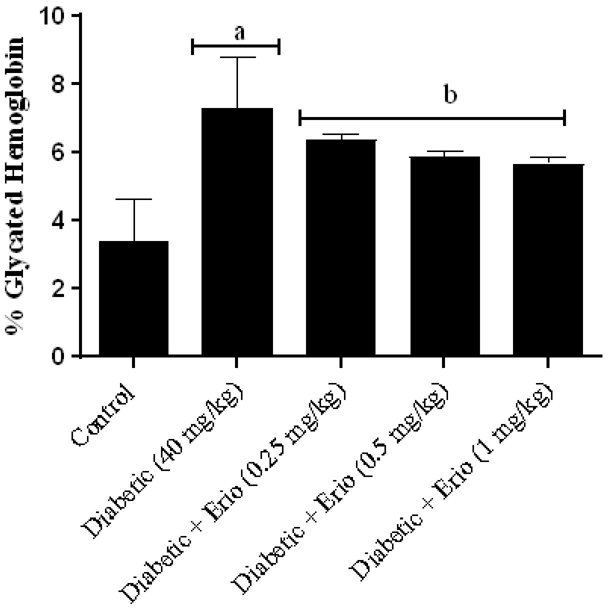

Figure 1. Effect of eriodictyol post-treatment on glycated hemoglobin in the blood of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Results are expressed as mean ± SD (n=10). Bar with a is significantly ($p<0.0001$) different from the control while bars with b are significantly ($p<0.0001$) different from the diabetic group. Erio: Eriodictyol.

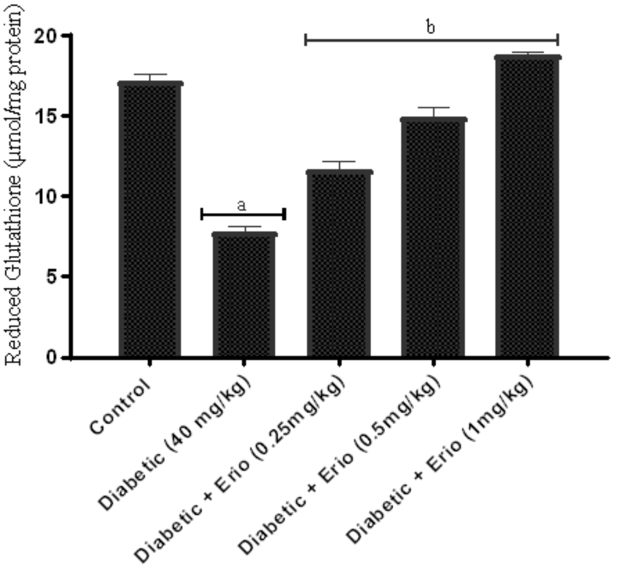

Figure 2. Effect of eriodictyol post-treatment on reduced glutathione level in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with a are significantly ($p<0.0001$) different from the control while bars with b are significantly ($p<0.0001$) different from the diabetic group. Erio: Eriodictyol.

Figure 10 shows the neuroprotective effect of eriodictyol against neuronal injury occasioned by

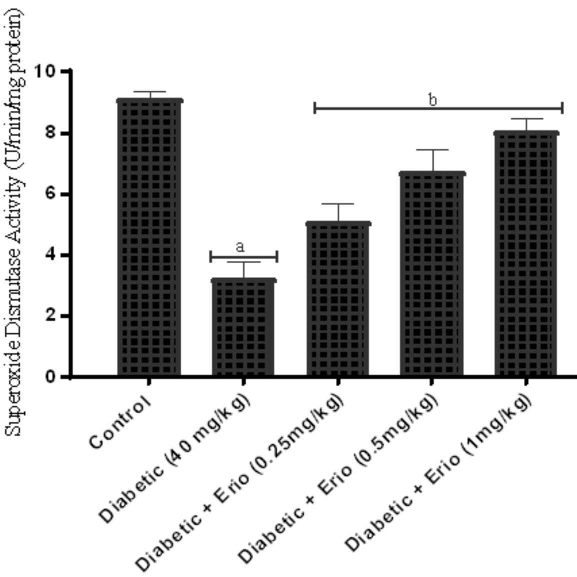

Figure 3. Effect of eriodictyol post-treatment on superoxide dismutase activity in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with a are significantly (p<0.0001) different from the control while bars with b are significantly (p<0.0001) different from the diabetic group. Erio: Eriodictyol.

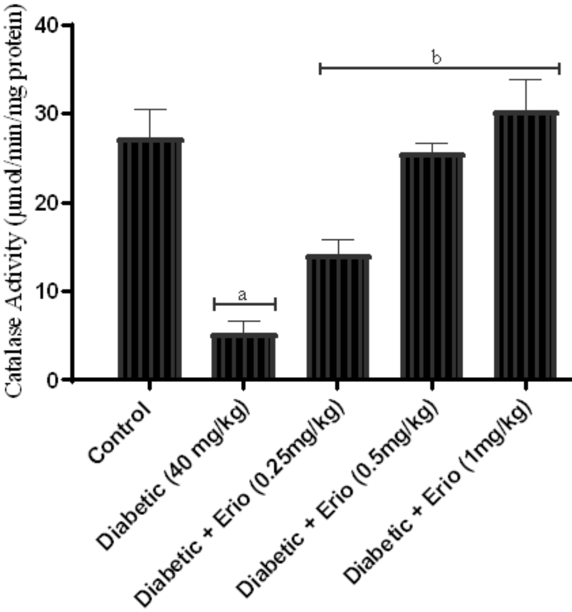

Figure 4. Effect of eriodictyol post-treatment on catalase activity in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with a are significantly (p<0.0001) different from the control while bars with b are significantly (p<0.0001) different from the diabetic group. Erio: Eriodictyol.

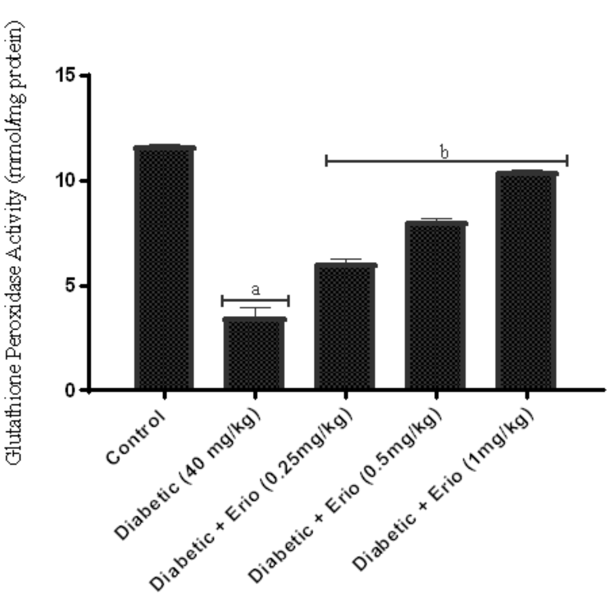

Figure 5. Effect of eriodictyol post-treatment on glutathione peroxidase activity in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with ^{a} are significantly (*p*<0.0001) different from the control while bars with ^{b} are significantly (*p*<0.0001) different from the diabetic group. Erio: Eriodictyol.

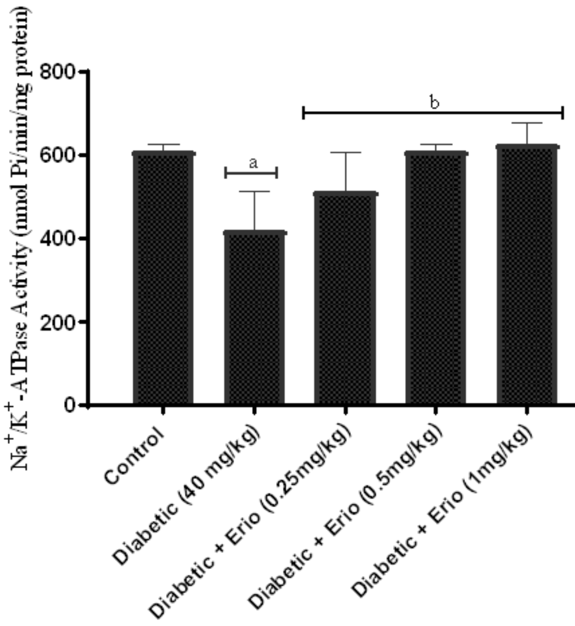

Figure 6. Effect of eriodictyol post-treatment on Na^+/K^+-ATPase activity in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with a are significantly (p<0.0001) different from the control while bars with b are significantly (p<0.0001) different from the diabetic group. Erio: Eriodictyol.

fructose/stz intoxication in hippocampus region of the diabetic rats, this was determined by H&E staining. In the positive control group, the hippocampal formation was severely depleted, degenerated neuronal cells in all the CA 1-4 were seen, poor structural organization of neuronal cells also appear scattered as well as noncompacted layers of the pyramidal cells were observed unlike the negative control group, where no pathological abnormalities were observed. However, eriodictyol post-treatment significantly (p<0.0001) ameliorated the fructose/streptozotocin-induced pathological changes by restoring normal hippocampal

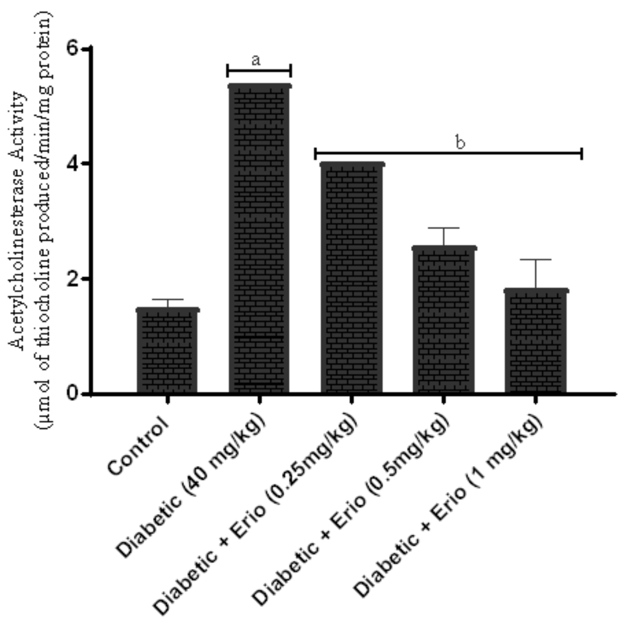

Figure 7. Effect of eriodictyol post-treatment on acetylcholinesterase activity in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with a are significantly (p<0.0001) different from the control while bars with b are significantly (p<0.0001) different from the diabetic group. Erio: Eriodictyol.

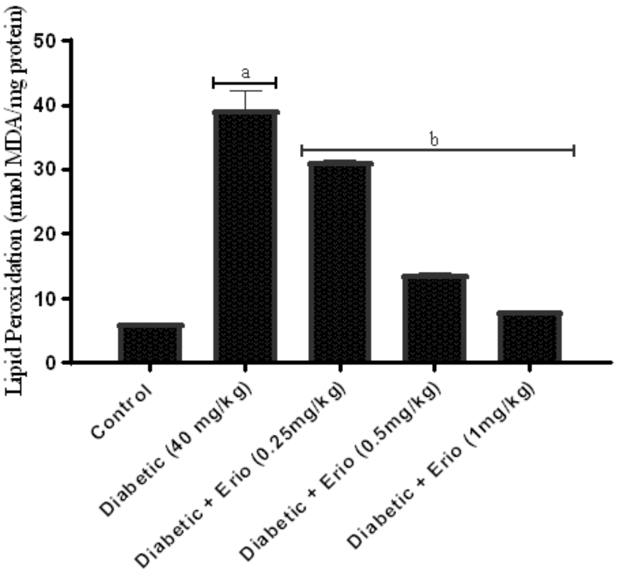

Figure 8. Effect of eriodictyol post-treatment on inhibition of lipid peroxidation in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with ^{a} are significantly (*p*<0.0001) different from the control while bars with ^{b} are significantly (*p*<0.0001) different from the diabetic group. Erio: Eriodictyol.

formation via regeneration of neuronal cells in all the CA 1-4, normal structural organization of neuronal cells, the layers appeared compact without scattering of neuronal cells, also, the pyramidal cells were compacted and normal.

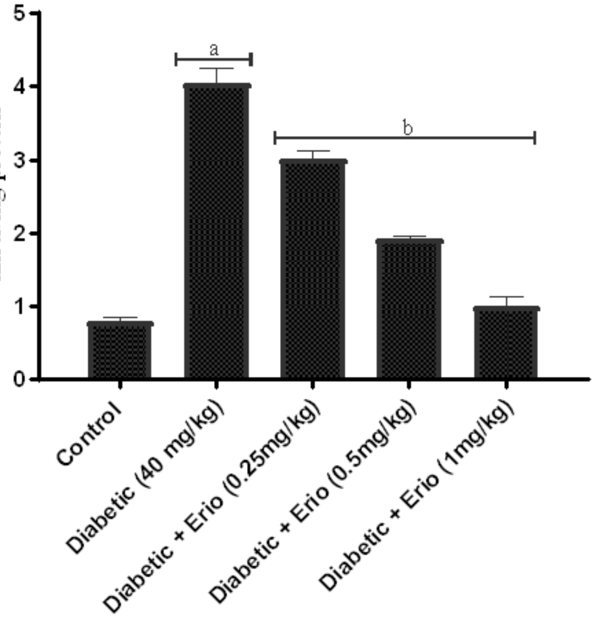

Figure 9. Effect of eriodictyol post-treatment on protein carbonyl level in the hippocampal regions of brain of fructose/streptozotocin-induced diabetic rats. Results are expressed as mean ± SD (n=10). Bars with a are significantly (p<0.0001) different from the control while bars with b are significantly (p<0.0001) different from the diabetic group Erio: Eriodictyol.

Diabetes mellitus is a prevalent and complex metabolic disorder resulting from impaired insulin secretion and/or action, leading to various macro and microvascular complications [28]. In diabetic neurological dysfunction, cerebral glucose uptake is significantly disrupted, and the brain is subjected to hyperglycemia [29]. As the most energy-demanding organ, the brain relies on glucose, consuming nearly 25% of the body's total glucose supply [30]. A consistent glucose supply is essential for maintaining normal brain metabolism and cognitive functions, but fluctuations in glucose levels can compromise these processes [31].

Flavonoids have recently garnered interest due to their potential health benefits, supported by studies in humans and animals, particularly because of their antioxidant properties [32]. The hydroxyl groups in flavonoids facilitate their antioxidant effects by scavenging free radicals or chelating metal ions, preventing the generation of radicals that damage biomolecules [33]. However, the neuroprotective efficacy of flavonoids is highly structure-dependent. This study evaluates the protective effects of eriodictyol on oxidative stress and histopathological alterations in the hippocampus brain region of fructose/streptozotocin-induced type-2 diabetic rats. The observed reduction in blood glucose levels following post-treatment with eriodictyol aligns with previous findings showing that dietary flavonoids lower blood glucose and enhance glucose-stimulated insulin secretion in diabetic rats [32]. Elevated HbA1c levels, associated with

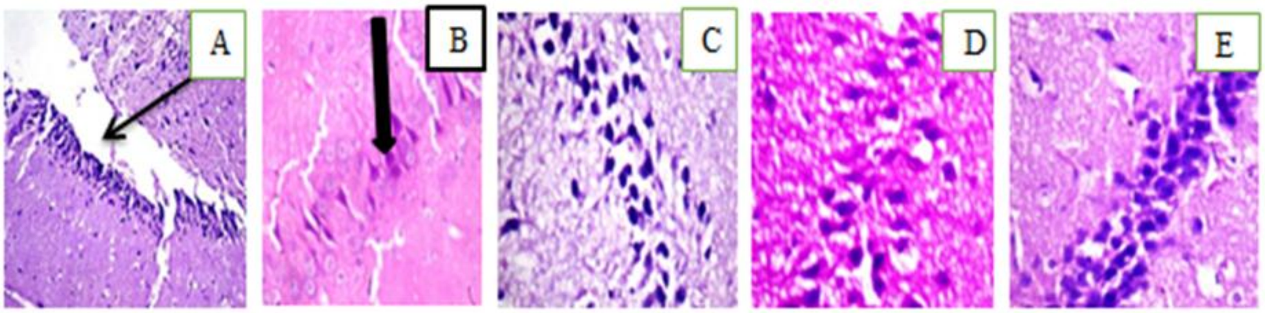

Figure 10. Representative of photomicrographs showing Hematoxylin and Eosin stained sections of Hippocampal region (Mag. x400). There were normal neuronal cells in all the CA 1-4, normal structural organization of neuronal cells, the layers appeared compact without scattering of neuronal cells, the pyramidal cells seen were also normal. (A) Control; (B) Fructose/Streptozotocin; (C) Diabetic + Erio (0.25 mg/kg); (D) Diabetic + Erio (0.5 mg/kg); (E): Diabetic + Erio (1 mg/kg); Erio: Eriodictyol.

hyperglycemia in diabetic rats, indicate poor reduced risks of diabetic complications. In this study, eriodictyol significantly reduced HbA1c, indicating its potential to mitigate hyperglycemia in type-2 diabetes, which could help prevent diabetes-related complications.

Numerous studies using various diabetes models suggest that hyperglycemia triggers oxidative stress, lipid peroxidation, and subsequent neuronal necrosis and apoptosis [34]. The brain, with its high aerobic demand and low antioxidant defenses, is particularly susceptible to oxidative damage, partly due to its low glutathione (GSH) levels [35]. GSH and its related enzymes play a crucial role in protecting cells against reactive oxygen species (ROS) by either directly neutralizing oxidants or acting as substrates for glutathione peroxidase [36]. The ameliorative effect of eriodictyol on antioxidant enzyme levels observed in this study aligns with previous findings that flavonoids enhance antioxidant defense in brain tissues of streptozotocin-induced diabetic rats.

Protein carbonyl content (PCO), a key marker of oxidative damage to proteins, was elevated in this study, indicating increased protein oxidation [37]. The decline in GSH may have contributed to protein denaturation and aggregation, particularly through thiol oxidation in diabetic rats' brains. However, post-treatment with eriodictyol reduced PCO levels, suggesting its ability to enhance GSH availability and mitigate protein oxidation.

Decreased acetylcholine levels in the diabetic rat brains which impairs neurotransmission and leads to cognitive deficits, were observed. The reduction in acetylcholinesterase (AChE) activity following eriodictyol treatment is consistent with previous reports, suggesting the flavonoid's potential to restore neurotransmission [38]. Similarly, the observed improvement in Na^+/K^+-ATPase activity after eriodictyol treatment supports its role in maintaining neuronal function and cellular homeostasis, aligning with previous findings.

In STZ-induced diabetic models, hippocampal damage affects both pyramidal neurons and astrocytes, leading to learning and memory impairments and neuronal structural abnormalities [39]. Severe damage including neuronal necrosis, vessel dilation, and neurofibrillary network deterioration was observed in the hippocampus of the diabetic rats in this study. However, Post-treatment with eriodictyol significantly improved these morphological alterations, demonstrating its neuroprotective potential.

In conclusion, this study highlights eriodictyol's antioxidant, neuroprotective, and antidiabetic effects, particularly in addressing oxidative stress and histopathological changes induced by type-2 diabetes. Eriodictyol's structural characteristics, especially the presence of a 3-hydroxy and 4-keto group but absence of a 2,3-double bond, may likely contribute to its high antioxidant and antidiabetic activities. These findings suggest eriodictyol therapeutic potential in preventing diabetes-associated complications and oxidative brain damage.

Conclusion

The results of this study demonstrate the potent antioxidant, neuromodulatory, and neuroprotective effects of eriodictyol in addressing oxidative stress and histopathological changes in fructose/STZ-induced type-2 diabetic rats. The findings suggest that eriodictyol's beneficial effects are likely mediated through its regulation of blood glucose transporters, enhancement of insulin secretion, antioxidant activity, promotion of pancreatic β-cell proliferation, reduction of insulin resistance, and mitigation of oxidative stress in the brain and muscle. Additionally, eriodictyol exhibited modulatory effects on energy metabolism. In conclusion, eriodictyol shows promise as a potential alternative therapy for developing effective neuroprotective and safe antidiabetic medications.

Contribution of authors

Agesin A. M., financed the experiment, performed the experiments, analyzed data and wrote the manuscript Akinmoladun. A. C, designed the experiment, provided administrative and professional supports and also reviewed the manuscript.

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Conflict of Interest

The authors declared no conflict of interest in the manuscript.

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Data Availability

The datasets in this article are not readily available due to technical limitations. Requests for access should be directed to the corresponding author via agesinanthony_monday@rugipo.edu.ng.

References

1. Antar, S.A.; Ashour, N.A.; Sharaky, M.; Khattab, M.; Ashour, N.A.; Zaid, R.T.; Al-Karmalawy, A.A. Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. Biomed. Pharmacother. 2023, 168, 115734.
2. Kropp, M.; Golubnitschaja, O.; Mazurakova, A.; Koklesova, L.; Sargheini, N.; Vo, T.T.K.S.; Thumann, G. Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications—risks and mitigation. EPMA J. 2023, 14 (1), 21–42.
3. Bellary, S.; Kyrou, I.; Brown, J.E.; Bailey, C.J. Type 2 diabetes mellitus in older adults: clinical considerations and management. Nat. Rev. Endocrinol. 2021, 17 (9), 534–548.
4. Álvarez-Rendón, J.P.; Murillo-Maldonado, J.M.; Riesgo-Escovar, J.R. The insulin signaling pathway a century after its discovery: sexual dimorphism in insulin signaling. Gen. Comp. Endocrinol. 2023, 330, 114146.
5. Dienel, G.A. Brain glucose metabolism: integration of energetics with function. Physiol. Rev. 2019, 99 (1), 949–1045.
6. Gupta, M.; Pandey, S.; Rumman, M.; Singh, B.; Mahdi, A.A. Molecular mechanisms underlying hyperglycemia associated cognitive decline. IBRO Neurosci. Rep. 2022, 14, 57–63.
7. Marinho, L.S.R.; Ikebara, J.M.; Higa, G.S.V.; de Lima Vasconcellos, T.H.; Inês, M.; Móvio, S.H.T.; Kihara, A.H. Function of the nervous system. Handb. Neural Eng. 2024, 17.
8. Fogwe, L.A.; Reddy, V.; Mesfin, F.B. Neuroanatomy, Hippocampus. StatPearls 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482171/
9. Abraham, W.C.; Jones, O.D.; Glanzman, D.L. Is plasticity of synapses the mechanism of long-term memory storage? NPJ Sci. Learn. 2019, 4 (1), 9.
10. Chandra, S.R. Alzheimer's disease: An alternative approach. Indian J. Med. Res. 2017, 145 (6), 723–729.
11. Chokron, S.; Kovarski, K.; Dutton, G.N. Cortical Visual Impairments and Learning Disabilities. Front. Hum. Neurosci. 2021, 15, 713316.
12. Khan, M.S.; Khan, S.; Khan, N.; Khan, A.S. Dietary sources, classification, biosynthesis, and mechanism of action of flavonoids in combating oxidative stress. Role Flavonoids Chronic Metab. Dis. 2024, 67–114.
13. Muscolo, A.; Mariateresa, O.; Giulio, T.; Mariateresa, R. Oxidative stress: the role of antioxidant phytochemicals in the prevention and treatment of diseases. Int. J. Mol. Sci. 2024, 25 (6), 3264.
14. Devi, S.; Kumar, V.; Singh, S.K.; Dubey, A.K.; Kim, J.J. Flavonoids: Potential candidates for the treatment of neurodegenerative disorders. Biomedicines 2021, 9 (2), 99.
15. de Lima, E.P.; Moretti Jr, R.C.; Torres Pomini, K.; Laurindo, L.F.; Sloan, K.P.; Sloan, L.A.; Barbalho, S.M. Glycolipid Metabolic Disorders, Metainflammation, Oxidative Stress, and Cardiovascular Diseases: Unraveling Pathways. Biology 2024, 13 (7), 519.
16. Obafemi, T.O.; Akinmoladun, A.C.; Olaleye, M.T.; Agboade, S.O.; Onasanya, A.A. Antidiabetic potential of methanolic and flavonoid-rich leaf extracts of Synsepalum dulcificum in type 2 diabetic rats. J. Ayurveda Integr. Med. 2017, 8 (4), 238–246.

17. Tapas, A.R.; Sakarkar, D.M.; Kakde, R.B. Flavonoids as nutraceuticals: a review. Trop. J. Pharm. Res. 2008, 7 (3), 1089–1099.
18. John, W.G.; Scott, K.W.; Hawcroft, D. Glycated haemoglobin and glycated protein and glucose concentrations in necropsy blood samples. J. Clin. Pathol. 1988, 41 (4), 415–418.
19. Beutler, E.; Mary, K.Y. Erythrocyte glutathione reductase. Blood 1963, 21 (5), 573–585.
20. Haque, M.E.; Asanuma, M.; Higashi, Y.; Miyazaki, I.; Tanaka, K.I.; Ogawa, N. Overexpression of Cu–Zn superoxide dismutase protects neuroblastoma cells against dopamine cytotoxicity accompanied by increase in their glutathione level. Neurosci. Res. 2003, 47 (1), 31–37.
21. Pandey, R.K.; Maranville, J.W.; Chetima, M.M. Tropical wheat response to irrigation and nitrogen in a Sahelian environment. II. Biomass accumulation, nitrogen uptake and water extraction. Eur. J. Agron. 2001, 15 (2), 107–118.
22. Kakkar, P.; Das, B.; Viswanathan, P.N. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys. 1984, 21 (2), 130–132.
23. Varshney, R.; Kale, R.K. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 1990, 58 (5), 733–743.
24. Levine, R.L.; Garland, D.; Oliver, C.N.; Amici, A.; Climent, I.; Lenz, A.G.; Stadtman, E.R. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990, 186, 464–478.
25. Ellman, G.L.; Courtney, K.D.; Andres Jr, V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961, 7 (2), 88–95.
26. Svoboda, P.; Mosinger, B. Catecholamines and the brain microsomal Na, K-adenosinetriphosphatase-I. Protection against lipoperoxidative damage. Biochem. Pharmacol. 1981, 30 (5), 427–432.
27. Day, M.J.; Bilzer, T.; Mansell, J.; Wilcock, B.; Hall, E.J.; Jergens, A.; Washabau, R. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. J. Comp. Pathol. 2008, 138, S1–S43.
28. Horton, W.B.; Barrett, E.J. Microvascular dysfunction in diabetes mellitus and cardiometabolic disease. Endocr. Rev. 2021, 42 (1), 29–55.
29. Blázquez, E.; Hurtado-Carneiro, V.; LeBaut-Ayuso, Y.; Velázquez, E.; García-García, L.; Gómez-Oliver, F.; Pozo, M.Á. Significance of brain glucose hypometabolism, altered insulin signal transduction, and insulin resistance in several neurological diseases. Front. Endocrinol. 2022, 13, 873301.
30. Magistretti, P.J.; Allaman, I. Brain energy and metabolism. Neurosci. 21st Century 2022, 2197–2227.
31. Cacciatore, M.; Grasso, E.A.; Tripodi, R.; Chiarelli, F. Impact of glucose metabolism on the developing brain. Front. Endocrinol. 2022, 13, 1047545.
32. Speisky, H.; Shahidi, F.; Costa de Camargo, A.; Fuentes, J. Revisiting the oxidation of flavonoids: Loss, conservation or enhancement of their antioxidant properties. Antioxidants 2022, 11 (1), 133.
33. Shen, N.; Wang, T.; Gan, Q.; Liu, S.; Wang, L.; Jin, B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chem. 2022, 383, 132531.
34. Papachristoforou, E.; Lambadiari, V.; Maratou, E.; Makrilakis, K. Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. J. Diabetes Res. 2020, 2020 (1), 7489795.
35. Lana, J.V.; Rios, A.; Takeyama, R.; Santos, N.; Pires, L.; Santos, G.S.; Lana, J.F. Nebulized Glutathione as a Key Antioxidant for the Treatment of Oxidative Stress in Neurodegenerative Conditions. Nutrients 2024, 16 (15), 2476.
36. Dumanović, J.; Nepovimova, E.; Natić, M.; Kuča, K.; Jaćević, V. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Front. Plant Sci. 2021, 11, 552969.
37. Colombo, G.; Reggiani, F.; Angelini, C.; Finazzi, S.; Astori, E.; Garavaglia, M.L.; Dalle-Donne, I. Plasma protein carbonyls as biomarkers of oxidative stress in chronic kidney disease, dialysis, and transplantation. Oxid. Med. Cell. Longev. 2020, 2020 (1), 2975256.
38. Bellavite, P. Neuroprotective potentials of flavonoids: Experimental studies and mechanisms of action. Antioxidants 2023, 12 (2), 280.
39. Salem, H. R., S Hanna, G., H Hassan, M., El-kotb, S., Rashad, S., Yassien, R. I., and Amer, G. S. Combined metformin and insulin therapy improves neurocognitive dysfunction in type 2 diabetic rat model via anti-inflammatory and antioxidant mechanisms. Physiol. Pharmacol. 2024, 28(2), 141-156.