JKIMSU, Vol. 10, No. 1, January-March 2021 **ISSN 2231-4261**

ORIGINAL ARTICLE

Effect of Fenofibrate on Hippocampal Insulin Resistance and Cognitive Function in a Rat Model of Type 2 Diabetes Mellitus

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Abstract:

Background: Hippocampal insulin resistance elicits decline in cognitive function and increased risk of neurodegeneration. Subsequently, Fenofibrate (FEN) has been used in metabolic disorders to treat hypercholesterolemia, hypertriglyceridemia and Insulin Resistance (IR), and may play a protective role against hippocampal insulin resistance in type 2 diabetic rats. *Aim and Objectives*: To study the effect of FEN on hippocampal insulin resistance and cognitive function in a rat model of type 2 diabetes mellitus. *Material and Methods:* Twenty male Wistar rats with an average weight of 200 ± 10 grams were randomly divided into four groups (n = 5/group). Induction of IR in subsets of the experimental rats was done by concurrent administration of High Fat Diet (HFD) and Streptozotocin (STZ) in a sub-set of the experimental rats. A dose of intraperitoneal injection of STZ-30 mg/kg was administered to the subjects for five consecutive days, while they were allowed to feed freely on high-fat diet for 90 days. The rats that received post-induction treatment were given 100 mg/kg FEN administered via intra-gastric gavage for 14 consecutive days. At the end of the experiment, an open-field test was conducted after which the rats were sacrificed and measurement of fasting plasma glucose and insulin as indices for insulin sensitivity, evaluation of enzyme activity and histological studies were carried out. *Results:* There was significant increase in the indices of IR, elevated anxiety level, reduced G6PD activity, elevated G6Pase activity, with numerous vacuolization in the pyramidal layer of hippocampal tissue in the HFD+STZ group compared to the control. However, there were significantly lowered indices of insulin resistance, increased G6PD activity, decreased G6Pase and reduced extent of vacuolization in the group that received post-induction FEN relative to untreated group. *Conclusion:* PPARα agonist activity of FEN plays a neuroprotective role on the hippocampal structure through different mechanisms of increasing insulin sensitivity, reducing anxiety, regulating metabolic enzyme activities and reducing features of neurodegenerationin HFD- and STZ-induced type 2 diabetic rats.

Keywords: Fenofibrate, Hippocampus, Insulin resistance, Neurodegeneration, Type-2-Diabetes

Introduction:

Insulin Resistance (IR) is a pathological condition in which the body cells lose sensitivity to insulin; a pancreatic hormone that promotes glucose uptake [1]. Metabolic disorder such as IR has been reported to contribute to the pathological consequences of Type 2 Diabetes Mellitus

(T2DM) and obesity [2]. The hippocampus has been found to be involved in behavioural coordination. Research has also revealed that hippocampal neurodegeneration associated with IR may be responsible for behavioural deficits including raised anxiety [3]. Structural and functional deficits in synaptic plasticity, coupled with impairments in a variety of behavioural tests of learning and memory, are observed in the hippocampus in rodent models of T2DM decline [4]. Indeed, Hippocampal IR (HIR) has also been associated with brain disorders such as anxiety and depression [5]. Experimental studies have further proven that HIR plays an important role in the mediation of synaptic plasticity and cognitive decline [2, 4].

Peroxisome Proliferator-Activated Receptors-alpha (PPARα) are transcriptional factors of the nuclear receptor super family which has been shown to regulate the genes expression involved in cell differentiation, immune function and metabolic homeostasis [6]. FEN is a PPARα agonist drug used in treatment of hypercholesterolemia and hypertriglyceridemia [7]. FEN is involved in the following processes which include; regulation of mitochondrial complex activity, glutamate homeostasis and cholinergic/ dopaminergic signaling in the neurons [8].

Material and Methods:

Ethics Review Committee for Animal Experiments of the University of Ilorin, Nigeria, approved the study with Clearance Number; UERC/ASN/2017/741 and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [9].

Chemicals:

FEN was procured from Bharat Parenterals Ltd, Gujarat, India. STZ was procured from Sigma-Aldrich Company (USA). HFD was compounded at Ace feed-mill, Ilorin, Nigeria. The diet was formulated according to the method of Akinola *et al*. [10]. Other materials and reagents were of analytical grade and procured locally.

Experimental Design:

Twenty male Wistar rats with an average weight of 200 ±10 g were sourced from animal holdings of the University of Ilorin. The rats were bred and maintained under standardized conditions of 12 hours day and night cycles, at the animal facility of the College of Health Sciences, University of Ilorin. The animals acclimatized at room temperature of 23 ± 5ºC for 14 days and were given rats feed ad libitum. The rats were randomly divided into two groups; the control (n=5) and test (n=15) groups. The control group received distilled water, while the test group was further divided into three subgroups which were administered normal diet and FEN (n=5) or HFD+STZ (n=5) or HFD+STZ+FEN (n=5). Induction of IR was done by concurrent administration of HFD and STZ in a sub-set of the experimental rats. The rats were fed on HFD throughout the experiment (90 days). STZ was dissolved in cold sodium citrate buffer (0.1M, pH 7.4) and 30 mg/kg body weight was administered intraperitoneally for 5 days. Fasting blood glucose level was estimated by using a smart glucometer (finetest glucometer, USA). A fasting blood glucose concentration of above 126 mg/dl was considered hyperglycaemic [10].

Blood glucose levels were evaluated weekly from tail vein blood using a handheld glucometer. FEN dissolved in distilled water was administered at a

dosage of 100 mg/kg for 14 days to the group of rats taking the treatment via intra-gastric gavage [11-12]. Subsequently, all the rats were fasted for 8 hours (10.00pm – 6.00am) and then euthanized using intraperitoneal injection of ketamine 10mg/kg [13]. Thoracotomy was performed to expose the heart for blood sample collection. The tissue samples for biochemical analysis were isolated, homogenized and cryo-preserved for further analysis. Whole body intracardial perfusion was performed on the subjects for histological analysis using 0.9% normal saline followed by 4% paraformaldehyde.

Open Field Test:

At the end of the experiment, the rats underwent an open field behavioral testing. The open field apparatus used was a wooden box (40 cm^3). The area of the open field, illuminated was divided into a 26 cm^2 central zone and a surrounding border zone. Each rat was placed at a corner of the open field at the beginning of a test and allowed to explore the open field for 8 min. Behavioral measures included number of lines crossed, total rearing number and total grooming time. The testing continued for 3 consecutive days [14].

Evaluation of Insulin Sensitivity:

Homeostatic Model for Insulin Resistance (HOMA-IR) is a method for assessing IR from fasting glucose and fasting insulin levels, with glucose concentration measured in mmol/L and insulin in µIU [15]. Blood samples were collected by cardiac puncture at the right ventricle into heparinized tubes and cryo-centrifuged (4ºC) at 3000rpm for 5 minutes. About 0.5ml of the plasma was then pipetted out for fasting glucose and insulin estimation. Fasting Plasma Glucose (FPG) was measured using glucose oxidase method according to Ambade *et al.* [16]. Fasting Plasma Insulin (FPI) levels was measured using rat insulin ELISA kit (Mercodia, Sweden), according to Akinola *et al*. (2018) [10]. HOMA-IR was calculated using the following formula:

$$\text{HOMA-IR} = \frac{(\text{FPI} \times \text{FPG})}{22.5}$$

Enzyme Activities:

The enzymatic tests were assayed with the supernatants from hippocampal homogenate, done at a temperature of 37°C. G6PD activity in hippocampus was assayed by the method of Kießling *et al.* [17]. While G6Pase activity was evaluated following the method of Barfell *et al.* [18].

Histological Analysis:

All the rats were fasted for 8 hours (10.00 pm – 6.00 am) and then euthanized using intraperitoneal injection of ketamine (10 mg/kg bodyweight). Thoracotomy was performed to expose the heart. The whole body was intracardially perfused with 0.9% normal saline followed by 4% paraformaldehyde. The brain was excised and rinsed in 0.25 M sucrose 3 times for 5 min each and post-fixed in 10% phosphate-buffer formalin for five days. Thereafter, the coronal sectioning of the brain was done to capture the hippocampus, tissues were further mounted and stained using Haematoxylin and Eosin (H and E) as described by Imam *et al*. [19]. The slides were examined under a light microscope (magnification, ×400).

Statistical Analysis:

Data were reported as mean ± SEM. All statistical analysis was performed, and graphs were drawn using GraphPad Prism 8 software (Graphpad software Inc. USA). Multiple comparisons were done using a one-way ANOVA with Tukey test. Significance was set at *p* less than 0.05 ($P < 0.05$).

Results:

FEN Increased Locomotion and Reduced Anxiety in Type 2 Diabetic Rats:

Anxiety level and locomotor activity of the rats were assessed in an open field apparatus. HFD+STZ and HFD+STZ+FEN groups showed statistically significant ($p < 0.05$) decrease in number of lines crossed (Fig. 1A), total rearing numbers (Fig. 1B) and total grooming time (Fig. 1C) compared to control. However, HFD+STZ+FEN group showed statistically significant ($p < 0.05$) increase in number of lines crossed, total rearing numbers and total grooming time compared to HFD+STZ group. There was no statistically significant difference between FEN and control.

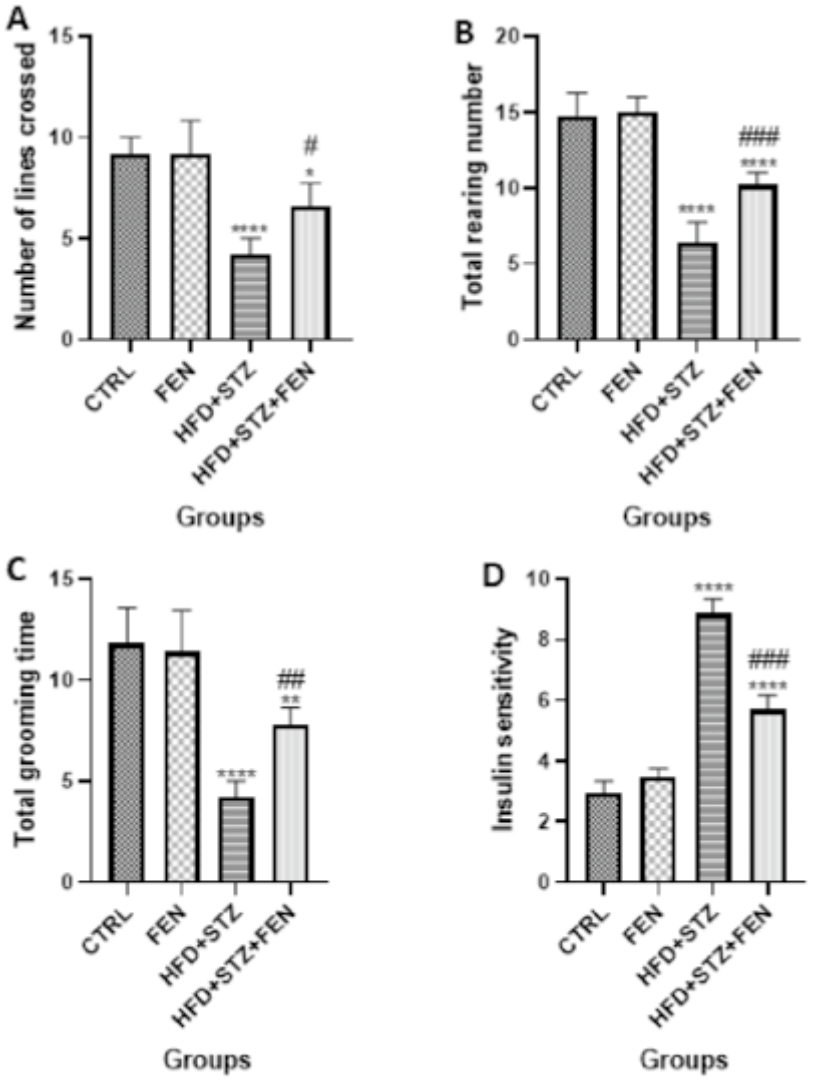

Fig. 1: Number of Lines Crossed (A), Total Rearing Number (B), Total Grooming Time (C), Insulin Sensitivity (D).

**P < 0.05 compared to control, #: P < 0.05 compared to HFD+STZ*

FEN Lowered Insulin Resistance in Type 2 Diabetic Rats:

Fasting plasma glucose and insulin levels were used to evaluate insulin activity, using HOMA-IR method (Fig. 1D). HFD+STZ and HFD+STZ+ FEN groups showed statistically significant ($p < 0.05$) increase in IR compared to control. Conversely, HFD+STZ+FEN group showed statistically significant ($p < 0.05$) decrease in IR compared to HFD+STZ. There was no statistically significant difference between FEN and control.

FEN Regulates Hippocampal Metabolic Enzymes Activity in Type 2 Diabetic Rats:

Metabolic function in the experimental rats was evaluated from G6PD and G6Pase activities. HFD+STZ and HFD+STZ+FEN groups showed statistically significant ($p < 0.05$) decrease in G6PD activity (Fig. 2A) and statistically significant ($p < 0.05$) increase in G6Pase activity compared to control. Contrarily, HFD+STZ+FEN group showed statistically significant ($p < 0.05$) increase in G6PD activity and notable decrease in G6Pase activity compared to HFD+STZ. There was no statistically significant difference between FEN and control. This result revealed that FEN improves metabolic activity in the diabetic rats.

FEN Attenuates Hippocampal Degeneration in Type 2 Diabetic Rats:

H&E stain histological assessment showed that the control and FEN groups appeared normal. The hippocampal parenchyma of HFD+STZ+FEN group showed the presence of darkly stained pyramidal shaped nuclei of the pyramidal neurons and lightly stained round shaped nuclei of the vesicular cell with little to no neurodegeneration. To oppose, the HFD+STZ group showed areas with neurodegeneration of the nuclei of the neurons of the hippocampus. Shrinkage and fragmentation of the nuclei leading to vacuolization of the pyramidal and vesicular cells of the hippocampus were the forms of neurodegeneration observed.

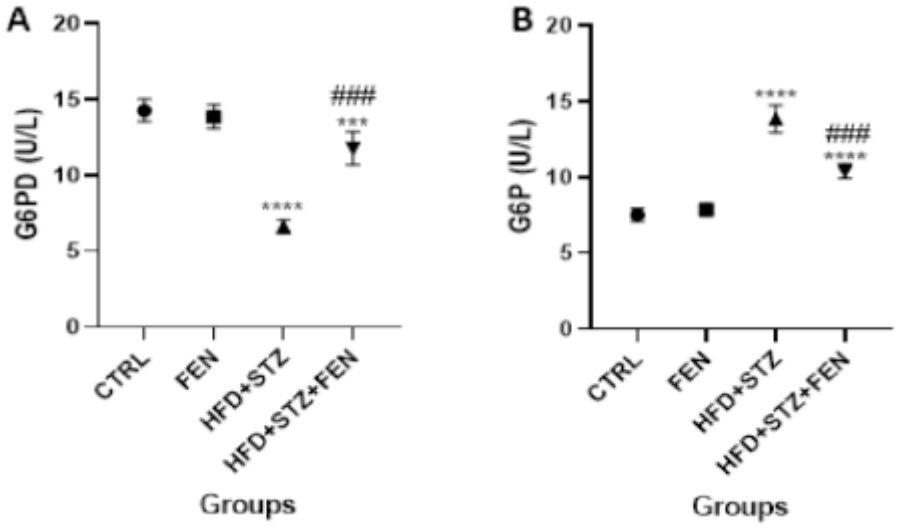

Fig. 2: G6PD Activity (A), G6P Activity (B).
**: P< 0.05 compared to control, #: P< 0.05 compared to HFD+STZ*

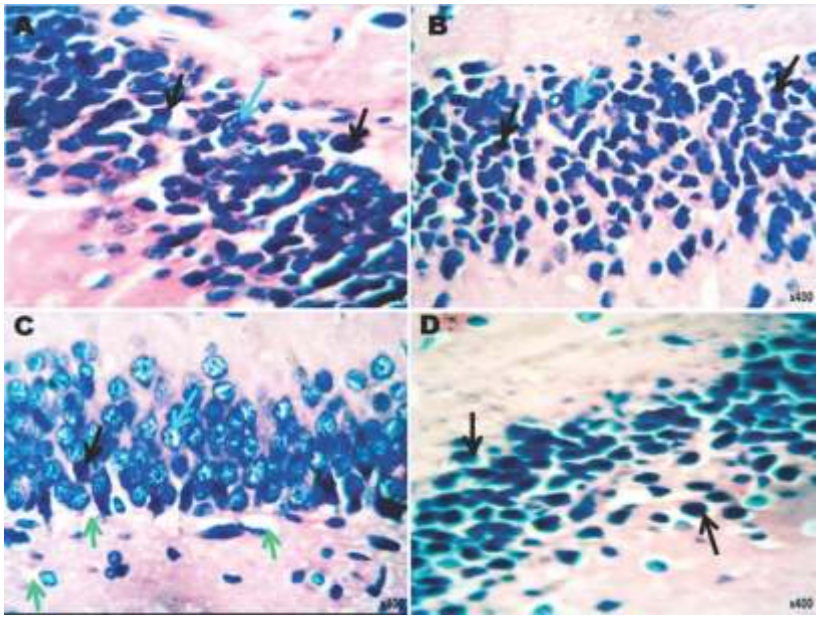

Fig. 3: H and E Stain Histological Assessment
A: control, B: FEN, C: HFD+STZ+, D: HFD+STZ+FEN. (black arrow: pyramidal neurons, blue arrow: vesicular cells, green arrows: degenerated neurons-vacuolization).

Discussion:

Various metabolic disorders including brain IR have been reported to be associated with T2DM [5]. Dysregulated insulin signaling may account for array of pathologies such as demyelination, dyslipidemia, neuroinflammation, and synaptic loss [20]. It has also been reported that PPARα alteration may result in metabolic disorders and neurodegeneration [8].

This study investigated the effect of FEN; a PPAR-α agonist on hippocampal degeneration due to IR resulting from HFD and STZ co-administration. In the open field test, the type 2 diabetic rats showed a significant decrease in the number of lines crossed, as well as total rearing number and total grooming time.

Subsequently, a reduced anxiety-like behavior in altered hippocampal morphology in female p75NTRexon IV- /- mice has been reported [14]. The result of the open field test suggests a locomotor deficit with anxiety in the type 2 diabetic rats which may be due to mitochondrial metabolic derangements in the brain centers responsible for production of ATP needed for locomotion, and loss of synaptic plasticity needed for cognitive formation [21-23]. However, PPARα agonist improves rearing number and grooming duration, reflecting a reduction in anxiety in the type 2 diabetic rats by increasing insulin sensitivity and glucose utilization [24]. Also, it was reported that pioglitazone and fenofibrate protected against behavioral impairments caused by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahyropyridine [25].

The animals that were administered with multiple intraperitoneal injection of low-dose STZ with long term intake of HFD presented severe IR. This is similar to the report of Akinola *et al.* (2018) [10] who induced IR using either HFD or high-fructose diet with multiple intraperitoneal injection of low-dose STZ. This may indicate a disruption in the signal propagating cascade of molecules collectively known as PI3K/Akt/mTOR signaling pathway [1, 26].

In this study, IR increased G6Pase activity in the hippocampus, which was accompanied by a decreased G6PD activity. G6PD is a metabolic enzyme involved in maintenance of cellular redox balance. The markedly reduced G6PD activity in the type 2 diabetic rats may be due to oxidative stress [27], resulting from low glucose level in the brain [28]. G6Pase is an enzyme that catalyzes gluconeogenesis, Glucose-6-phosphatase-β (G6Pase-β) couples with the glucose-6-phosphate (G6P)-transporter to hydrolyze G6P to glucose in the terminal stages of glycogenolysis in the brain [29]. The increased G6Pase activity in the hippocampus may be due to starvation of the neurons requiring gluconeogenesis [29]. However, treatment with FEN improved insulin sensitivity by decreasing G6Pase activity and increasing G6PD activity. These current findings suggested that an imbalance in glucose metabolic pathways is an important factor to hippocampal oxidative damage and behavioural deficit in T2DM.

H and E stain histological analysis showed degeneration of the nuclei of the neurons of the hippocampus in the forms of shrinkage and fragmentation of the nuclei leading to vacuolization of the pyramidal and vesicular cells. The observed neurodegenerative features may be due to distortion of the cascade of neurochemical signaling (glutamatergic/GABAergic) pathways in the hippocampus [21-22]. Interestingly however, FEN substantially reduced the extent of neurodegeneration in type 2 diabetic rats. This histoarchitectural analysis served as complementary evidence to the analysis on functional indices and enzymatic assays. Although, FEN was reported to have interspecies differences in PPAR-α agonist effects which occurred in both rodents and humans, because contrary to the rodents, there were no direct effects of fibrates on adipose tissue present in the human subjects [30].

Conclusion:

This study provided the evidence that, FEN plays a neuroprotective role on hippocampal degeneration in HFD- and STZ-induced type 2 diabetic rats through its effect on behavioral parameters, enzyme activity and hippocampal histoarchitectural modifications.

Acknowledgements:

The authors appreciate the members of staff of the Faculty of Pharmacy and the Departments of Histopathology and Anatomy, University of Ilorin, Nigeria, for their technical support during this work.

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How to cite this article:
Olumayowa KI, Stephen OA, Olushola OO, Nafeesa MA, Akeem AO. Effect of Fenofibrate on Hippocampal Insulin Resistance and Cognitive Function in a Rat Model of Type 2 Diabetes Mellitus. *J Krishna Inst Med Sci Univ* 2021; 10(1):75-83

Submitted: *01-Oct-2020* ***Accepted:*** *19-Dec-2020* ***Published:*** *01-Jan-2021*