



Original article

Localised streptozotocin-induced structural and cognitive changes in the hippocampal cornu ammonis 1 (CA-1) neurons and mitigating effects of *Zingiber officinale*

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ABSTRACT

Background: The study was done to establish the neurotoxic effect of intracerebroventricular streptozotocin (ICV STZ)-induced brain insulin resistance, and also considered *Zingiber officinale* (*Z. officinale*) mechanism of action in ameliorating behavioural, molecular and histopathological changes associated with ICV STZ-induced brain insulin resistance in Wistar rat model.

Method: 32 male Wistar rats weighing between 160 and 200 g were grouped into 4 ($n = 8$) labelled A-D and housed in standard seized home plastic cages at 23 ± 1 °C. Group-A (control) received a single ICV injection of normal saline, Group-B received 300 mg/kg of *Z. officinale*, Group-C received single dose of ICV STZ at 3 mg/kg and Group-D received single dose of ICV STZ at 3 mg/kg with 300 mg/kg of *Z. officinale*. Administration of *Z. officinale* extract was done orally and for 21 consecutive days. Values were represented in graphs and table, and analysed using GraphPad Prism 6.0 software.

Results: Organ weight revealed a drastic loss, which was not significant following 3 mg/kg of ICV STZ. Also, ICV STZ significant reduced and increased rat performance in open arm entry (OAE) and closed arm duration (CAD) respectively, with an obvious insignificant reduction of rat spontaneous alterations (SA) performance as well as the relative expression of insulin receptor substrate (IRS) gene in the brain when compared with control and other groups. The relative expression of Beta-secretase 1 (BACE-1) and glycogen synthase kinase 3-beta (GSK 3 β) gene significantly increased in ICV STZ treated rats. Furthermore, photomicrographs revealed scattered layers of pyramidal cells, with chromatolysed and degenerating neurons in the cornu ammonis 1 (CA-1) region of the hippocampus. Similarly, accumulation of amyloid-beta was seen in the CA-1 region, which correlates the loss of neuronal cells and neurobehavioural deficit.

Conclusion: However, *Z. officinale* was capable of mitigating this effect by enhancing the antioxidant defense system, which possibly inhibited the activities of free radical or reactive oxygen generation and subsequently suppressed GSK 3 β and BACE-1 gene expression.

1. Introduction

Insulin resistance is a progressive metabolic disorder, characterized

by compromised blood glucose-lowering effect of circulating or injected insulin on blood glucose as well as the reduction in the metabolic response of target cells to insulin in an organism (Czech, 2017; Ovalle

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and Azziz, 2002). Several studies have shown that Insulin dysregulation acts in a variety of ways, accounting for morphological and physiological alteration such as decreased cortical glucose utilization (Fu et al., 2013; Wilcox, 2005), oxidative stress (Tangvarasittichai, 2015), formation of advanced glycated proteins (Song and Schmidt, 2012), increased neurofibrillary formation (C. Benedict and Grillo, 2018) and increased β -amyloid aggregation through inhibition of insulin-degrading enzyme (Grossman, 2003). There is growing evidence that insulin resistance is highly involved in cognitive decline (Arnold et al., 2018; C. Benedict and Grillo, 2018; Ferreira et al., 2018; Shankar et al., 2008). Insulin and insulin receptors has been shown to be widely distributed in the brain (especially in the hippocampus and hypothalamus) and the alteration of insulin receptor signaling often leads to amyloid β ($A\beta$) formation and tau protein hyperphosphorylation (Banks et al., 2012; Crane et al., 2013; Shankar et al., 2008; Suzanne, 2017).

Glycogen synthase kinase 3 beta (GSK3- β) is a serine/threonine protein kinase, which is known as a major player in the pathogenesis of several chronic diseases including neurodegenerative disorders such as Alzheimer's disease (AD) (Llorens-Marín et al., 2014). This protein is highly expressed in adult rodent brain, mainly in hippocampus, neocortex and cerebellum (Llorens-Marín et al., 2014). In normal circumstance, insulin suppresses GSK3- β activity through the insulin receptor/insulin receptor substrate (IRS)/PI3K/Akt pathway (Saltiel and Kahn, 2001). Perturbation of this pathway (as occurs in type II diabetes) contributes to the aetiopathogenesis and progression of hyperglycaemia in type II diabetes (Eldar-Finkelman et al., 1999) and also directly enhances $A\beta$ generation and tau hyper-phosphorylation in AD (Llorens-Marín et al., 2014; Phiel et al., 2003). Thus, GSK3- β is a common mechanistic link between type II diabetes and AD and thus a putative target for their treatment. In furtherance, beta-secretase 1 (BACE-1) is primarily and abundantly expressed by neurons in brain (Vassar and Cole, 2007), and when unregulated, it can alter synaptic plasticity correlating amyloid precursor protein (APP), neuregulin-1 (Nrg1), seizure-related gene 6 (Sez6), and Jagged-1 (Jag1) cleavage, thus, it is crucial for $A\beta$ generation in the brain (Causevic et al., 2018; Das and Yan, 2017). Inhibition of GSK3- β protein has been reported to deplete BACE-1-mediated cleavage of APP, Nrg1, Sez6 and Jag1 through a NF- κ B signaling-mediated mechanism (Llorens-Marín et al., 2014), indicating that GSK3- β inhibition will reduce the pathology of $A\beta$ plaque in the brain.

Zingiber officinale (Z. officinale) is a dietary and herbaceous plant, and a major phenolic constituent of various plants belonging to the Zingiberaceae family (Grieb, 2016). Its rhizomes are very medicinal having 6-gingerdiol, 8-gingerol, 10-gingerdione and 6-shogaols, as its active phytochemical constituents (White, 2007). Z. officinale has been reported to exhibit several pharmacologic activities such as antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, anti-apoptotic, and hepatoprotective properties which suggest its possible protective effect against intracerebroventricular streptozotocin (ICV STZ) neurodegenerative manifestations (Halawany et al., 2017; Mbaveng and Kuete, 2017; Shahrajabian et al., 2019). Thus, the essence of this research is to establish the neurotoxic effect of ICV STZ-induced brain insulin resistance, and also considered Z. officinale extract mechanism of action in ameliorating behavioural, molecular, and histopathological changes associated with ICV STZ-induced brain insulin resistance in the Wistar rat model. Our specific objectives are to evaluate the neurobehavioral changes of animals, examine the histo-architecture of their hippocampus and ascertain the level of IRS, BACE-1 and GSK3- β gene expression in the hippocampus.

2. Materials and methods

2.1. Animals care, handling, and treatment

Thirty-two (32) adult male Wistar rats weighing between 160 and 200 g were gotten from the animal housing facility, Babcock University,

Ilishan-remo, Ogun state and housed in the Institutional Animal Holding Facility using standard seized home cages under suitable environmental conditions (at 23 ± 1 °C) throughout duration of the treatment. Distilled water and pelletized feed were given to the Wistar rats daily. Filtered wood shavings that were been changed every 2 days to avoid the build-up of toxic substances were used as beddings for the Wistar rats. Experimental rats were divided into 4 groups ($n = 8$) to avoid overcrowding, labelled A-D. Group A (control) received a single icv injection of normal saline, Group B received 300 mg/kg/ of Z. officinale Fouda and Berika, 2009, Group C received a single dose of icv STZ at 3 mg/kg and Group D received a single dose of icv STZ at 3 mg/kg with 300 mg/kg/ of Z. officinale. Administration of Z. officinale extract was done orally and day for 21 consecutive days. All experimental activities involving animal use, handling, treatment procedure, and management were carried out in compliance with ethics and standard institutional research practices. Approval was obtained from Babcock University Health Research Ethics Committee (approval number BUHREC 811/18).

2.2. Extract preparation and administration

The extraction procedure was done according to Fouda & Berika, (Fouda and Berika, 2009). Briefly, Z. officinale was gotten from Ilishan main market and then processed in Babcock University. It was blended and mixed with ethanol for 48 h to produce ethanol extract. The extract was measured for administration using an oral cannula.

2.3. STZ procurement, preparation and administration

STZ was purchased from Sigma-Aldrich, St Louis, MO, USA, dissolved in saline solution and kept at a cool temperature of -16 °C. STZ for inducing insulin resistance was administered to the rat brain intracerebroventricularly at 3 mg/kg in 2 μ l of 0.9% normal saline (vehicle), with the aid of a stereotaxic instrument (constructed by a neurobiologist in our laboratory (see Fig. 1)) and Hamilton syringe.

2.4. Pre-surgical procedure

Before the surgery, an anesthesia cocktail containing 0.5 ml of xylazine and 10 ml of ketamine was prepared to make the rat unconscious. Heating pads were turned on at normal body temperature to maintain body temperature and basal metabolism of the rat after surgery. A stereotaxic atlas was used to determine the exact location in the brain to inject and release STZ. Based on the atlas and location of the lateral ventricle in a rat, the coordinates were 0.72 mm anterior-posterior, 1.0 mm lateral-medial and 4.0 mm depth (Paxinos and Watson, 2006; Paxinos et al., 2009). Stereotaxic instrument was thoroughly cleaned with methylated spirit.

2.5. Surgical procedure

Once the rat was unconscious, it was mounted on the stereotaxic instrument and fixed in a rigid position. Afterward, the head was cleaned with methylated spirit and an incision was made on the head of the rat anterior-posteriorly. The cranium was cleaned with hydrogen peroxide to remove the surrounding fatty tissue and provide a clear view of the area. The point at which the cranium was to be drilled was located using the calculations done with the stereotaxic atlas. This point was then drilled through the cranium and a needle was used to bore the meningeal layer. Hamilton syringe containing STZ was then mounted and used to administer it into the hippocampus through the drilled hole. STZ was left to disperse in the cerebrospinal fluid of the left lateral ventricle and the syringe was removed. This is a surgical procedure that was performed with precision and care to get accurate results. (Halawany et al., 2017; Kraska et al., 2012; Sharma and Gupta, 2001).



Fig. 1. Pictures showing the Icv STZ administration procedure.

2.6. Post-surgical procedure

The area was cleaned with methylated spirit and the head was sutured. At this point, antibiotic paste (penicillin antibiotic paste) was applied properly to the surgical area. The rat was then placed on the heating pad and left to regain consciousness (Grieb, 2016). The rats were then carefully taken to their respective cages to ambulate and heal properly. While treating with *Z. officinale* extract, the rats were handled under suitable environmental conditions at $23 \pm 10^\circ\text{C}$, and carefully administered with *Z. officinale* extract. Also, penicillin antibiotic paste was rubbed on the surgery site twice a day (morning and evening), until complete healing took effect.

2.7. Neurobehavioural testing

Both neurobehavioural tests (elevated plus maze and Y-maze) were conducted on day 22.

Elevated plus maze (EPM): The height of the EPM used in this experiment was approximately 50 cm. During the test, rats were placed in the central area, facing an open arm and allowed to probe the elevated maze for 5 min and a valid entry was considered and recorded when the four paws of the rats enter the arms. Open arm entry closed arm duration of each rat was recorded for statistical analysis (Yuan et al., 2019).

Y-maze: Y-maze spontaneous alternation test is a neurobehavioural test carried out to examine the willingness of experimental animals (such as Wistar rats) to explore new environments. It consists of 3 (A, B and C) arms diverging from the central point, and rats were firstly put at the center and allowed to probe the apparatus in a six-minute test session. Each rat activity for six minutes was recorded (Yuan et al., 2019).

2.8. Tissue preparations

24 h after neurobehavioural test, all animals were sacrificed through cervical dislocation method, and rat brain weights were measured with the use of a digital weighing balance. Two animals from each group were perfused with 10% formal-saline and their brain were carefully excised, and fixed in 10% formalin for histological and immunohistological examination while the others animal in the same group were not perfused, rather their brain were homogenized and used for GSK3- β and BACE-1 gene expression analysis.

2.9. GSK3- β and BACE-1 gene expression assay

Gene expression assay was done according to Omotuyi, et al., (Omotuyi et al., 2018). Briefly, RNA was isolated from the thin slice coronal section of the hippocampus using TRIzol Reagent

(ThermoFisher Scientific). DNA contaminant was removed following DNase I treatment (ThermoFisher Scientific) according to manufacturer's protocol. Purified DNA-free RNA was converted to cDNA immediately using ProtoScript®First Strand cDNA Synthesis Kit (NEB). PCR amplification was done using OneTaq® 2X Master Mix (NEB).

2.10. Statistical analysis

Values were represented as the mean $\pm$ SEM in graphs and table. The significant differences were defined with one-way ANOVA, followed by Newman-Keuls post hoc test, analyzed using the Graphpad Prism 6.0 software. The confidence interval was placed at 95% and considered a $p < 0.05$ as statistically significant. Prepared histological and immunohistological slides were viewed using a microscope connected to a computer with a digital camera.

3. Results

3.1. Brain weight

Fig. 2 shows the brain weight of the animals across the groups. Observable decrease was seen in group-C (1.112 ± 0.01689) that was administered with a single dose of icv STZ at 3 mg/kg, when compared with group-A (1.176 ± 0.01824). However, a slight increase was observed in group-D (1.165 ± 0.01841) that was administered with a single dose of icv STZ at 3 mg/kg with 300 mg/kg of *Z. officinale*, when compared with group-C. The brain weight of group-B was slightly higher (1.216 ± 0.02485), when compared with group-A (control).

3.2. *Z. officinale* improved the working memory performance of type-II diabetic rat model

As seen in Table 2, Icv STZ -induced brain insulin resistance rat showed significant deficits on memory and cognitive function based on our neurobehavioural findings. Thus, we evaluated the attenuating potential of *Z. Officinale* in the working memory on type-II Diabetic rat, by performing EPM and Y-maze test. EPM parameter scored following type-II Diabetes induction (group-C), revealed a significant decreases and increases in open arm entry (OAE) and closed arm duration (CAD) respectively when compared with group-A (control). Also, Y-maze test result showed that the spontaneous alternation (SA) activity of group-C rat exhibited an obvious decrease which was not significant when compared with group-A. These finding suggest the underlining deficits of working memory following a single dose of icv STZ at 3 mg/kg. Group-B revealed no significant difference in OAE and CAD of EPM, and SA activity of Y-maze test when compared with group-A. However,

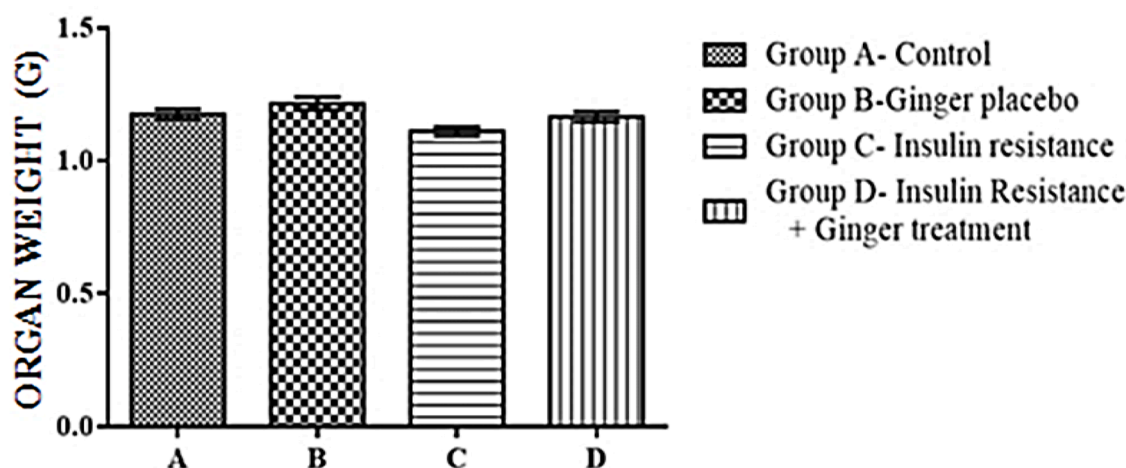


Fig. 2. Bar chart showing the changes in brain weight of control and treated groups. Data obtained are in mean $\pm$ SEM. There were no statistical significant difference in-between groups.

Z. officinale effectively prevented the memory loss, as group-D showed significant increase in OAE with an obvious depletion in CAD of EPM, and a notable increase in SA of Y-maze, when compared with group-C.

3.3. *Z. officinale* modulates the expression of genes involved in glycogen synthase kinase 3 beta (GSK3- β) signaling pathogenesis leading to neurodegeneration

In this study, the expression of GSK3- β and associated gene implicated in neurodegenerative diseases were investigated using reverse transcriptase polymerase chain reaction (RT-PCR) to ascertain their involvement in the pathogenesis of neurodegeneration, and possible modulatory roles of *Z. officinale* extract. Data shows that a single dose of icv STZ at 3 mg/kg (group-C (155.5856 $\pm$ 13.500)) resulted in a notable down-regulation of insulin receptor substrate (IRS) when compared with

control groups (group-A (199.1692 $\pm$ 13.400)). Group-B (308.7905 $\pm$ 16.000) was significantly high when compared to control. However, interventions with *Z. officinale* extract in the pathogenic model (group-D (184.0087 $\pm$ 13.500)) obviously increased the expression of IRS when compared with group-C (see Fig. 3a). Also, GSK3- β was up regulated in group-C (131.8994 $\pm$ 11.5) when compared to the control (group-A (98.58488 $\pm$ 9.800)) and other experimental groups (group-B (89.83674 $\pm$ 8.900)). Treatment with *Z. officinale* extract in the pathogenic model (group-D (117.0176 $\pm$ 10.500)) obviously depleted GSK3- β gene expression (see Fig. 3b). Fig. 3c revealed the expression of BACE-1 gene across experimental groups. BACE-1 gene expression in groups-C (98.8875 $\pm$ 9.600) was up regulated significantly when compared to control (46.8188 $\pm$ 6.000). Group-B (87.32014 $\pm$ 8.700) was significantly high when compared to control. However, Treatment with *Z. officinale* extract (group-D (82.48808 $\pm$ 10.500)) was effective as there

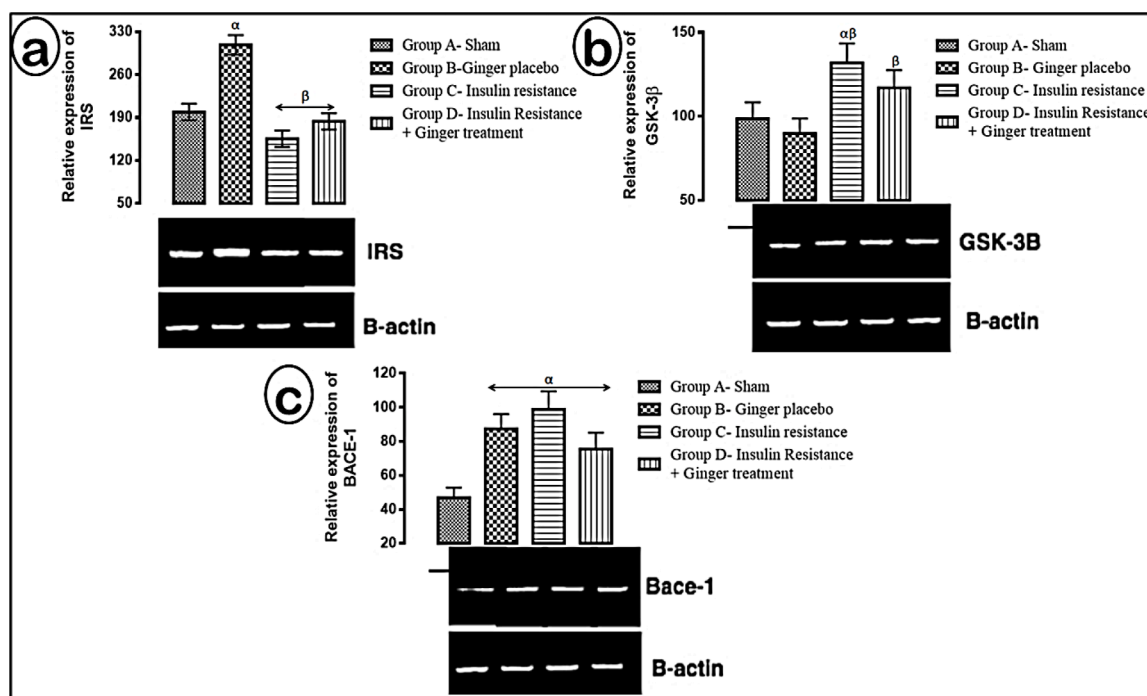


Fig. 3. Bar chart showing the Relative Expression of (a) Insulin Receptor Substrate (IRS) gene; (b) Glycogen Synthase Kinase 3-Beta (GSK 3 β) gene; (c) Beta Secretase (BACE 1) isolated using mRNA from the hippocampus. Data obtained are in mean $\pm$ SEM. α = significantly different from group-A; β = significantly different from group-B ($p \leq 0.05$).

was a notable decreased in the expression of BACE-1 gene after being treated with *Z. officinale* extract when compared compare to group-C.

3.4. Structural alteration and Nissl profiling in the hippocampus, and the mitigating effects of *Z. officinale* extract

Histopathological studies have been increasingly used to identify specific mechanism that mediates behavioural changes in neurodegenerative diseases (NDDs) and this provides a good platform for seeking effective therapeutic intervention (Cummings and Zhong, 2006). Following behavioural consideration, the general cyto-architecture of the hippocampus was examined across treatment groups using haematoxylin and eosin methods. Furthermore, rough endoplasmic reticulum as well as ribosomal integrity was investigated using crysl fast violet to determine the underlying mechanisms involved in the therapeutic activities of *Z. officinale* extract. Under light microscope, the presentation of the cornu ammonis 1 (CA-1) in hippocampus of rat brains was captured because they constitute large pyramidal neurons with efferent and afferent connection with other brain part. The morphological presentation of the CA-1 region in control (Group-A) reveals a well-arranged pattern of the pyramidal neurons with long axons, and similar finding was seen in Group-B. As seen in Fig. 4, there was dispersion in the arrangement of pyramidal neurons, and degenerating features were seen in Group-C. Also, cresyl violet stain demonstrating Nissl substances in tissue sections were not properly expressed in Group-C (see Fig. 5). However, therapeutic treatment using *Z. officinale* (Group-D) elicited putative properties as there was well-arranged pyramidal cell with long axons in the CA-1 region, and also a clear demonstration of Nissl substance in tissue sections, very similar to those of control and Group-B.

3.5. *Z. officinale* extract reverse amyloid plaque formation in the hippocampus

Plaques formation is one of the major hallmarks of dementia and associated diseases. In this present study, the formation of plaques and amyloid β expression was analyzed in the hippocampal CA-1 region

using anti-rat amyloid β immunostaining to probe neurons for amyloid plaques formation. As seen in Fig. 6, control group (group-A) and Group-B show mild reactivity to anti-rat amyloid β with no formation of amyloid plaques. Contrastingly, Group-C showed intense reactivity to anti-rat amyloid β with aggregation of amyloid plaques. However, the hippocampal CA-1 region of Group-D showed low reactivity of anti-rat amyloid β when compared with Group-C, and there was no formation of amyloid plaques and amyloid β density was quite similar to that of control.

4. Discussion

Hippocampal neuronal dysfunction caused due to ICV STZ-induced brain insulin resistance (type-II Diabetes) has not been fully explore and may play a vital role in long-term neurological deficits (Spinelli et al., 2019). In this present study, the neurotoxic effect of ICV STZ-induced brain insulin resistance was examined, and *Z. officinale* extract mechanism of action in ameliorating behavioural, molecular and histopathological changes associated with ICV STZ-induced brain insulin resistance in Wistar rat model. The organ (brain) weight reported in this study revealed a slight loss of weight in the diabetic group although, not significant when compared to other experimental groups. The loss of brain weight noticed in this study may be due to the diffusion/dispersion STZ in the brain, and reduced uptake of brain glucose. Asagba, et al., finding have provided evidence of decreased brain weight, which is similar to our result (Asagba et al., 2019). Treatment with *Z. Officinale* extract following ICV STZ-induced brain insulin resistance, obviously improved the brain weight as seen in Fig. 2.

Table 1

The brain comprises neurons that communicate via synaptic ending to attain various functions, which could manifest in the behavioural outcome of an organism. However, the brain is very sensitive to oxidative stress, which is as result of the generation of reactive oxygen species (ROS), leading to several neural deteriorating processes in diabetic condition (Sangi and Jalaud, 2019). In this study, ICV STZ-induced brain insulin resistance showed significant deficits in memory and cognitive function based on our neurobehavioural findings (Table 2). We reported

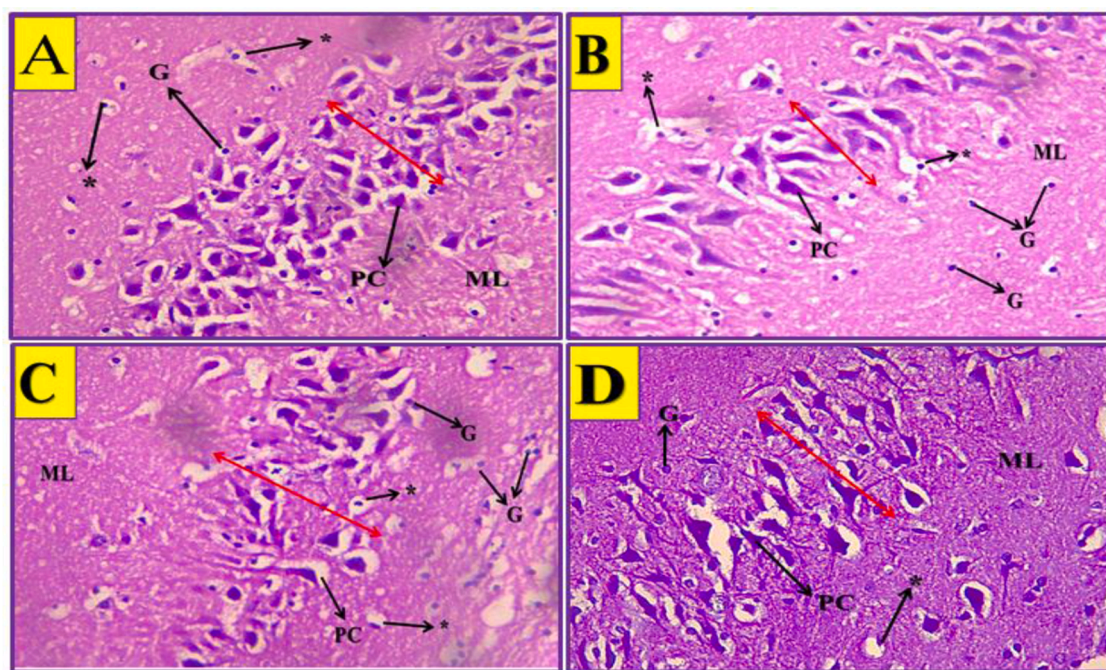


Fig. 4. Photomicrographs of sections showing the cytoarchitecture of Cornu Ammonis 1 in hippocampus of the brains stained with Hematoxylin and Eosin [X400]. ML (Molecular Layer); PC (Pyramidal Cells); G (Glial cells); * (Deteriorated Glial Cells); Double head arrow: Pyramidal Layer (Polarized or depolarized). A (icv Saline); B (*Z. officinale* 300 mg/kg); C (icv STZ 3 mg/kg); D (icv STZ 3 mg/kg + *Z. officinale* 300 mg/kg).

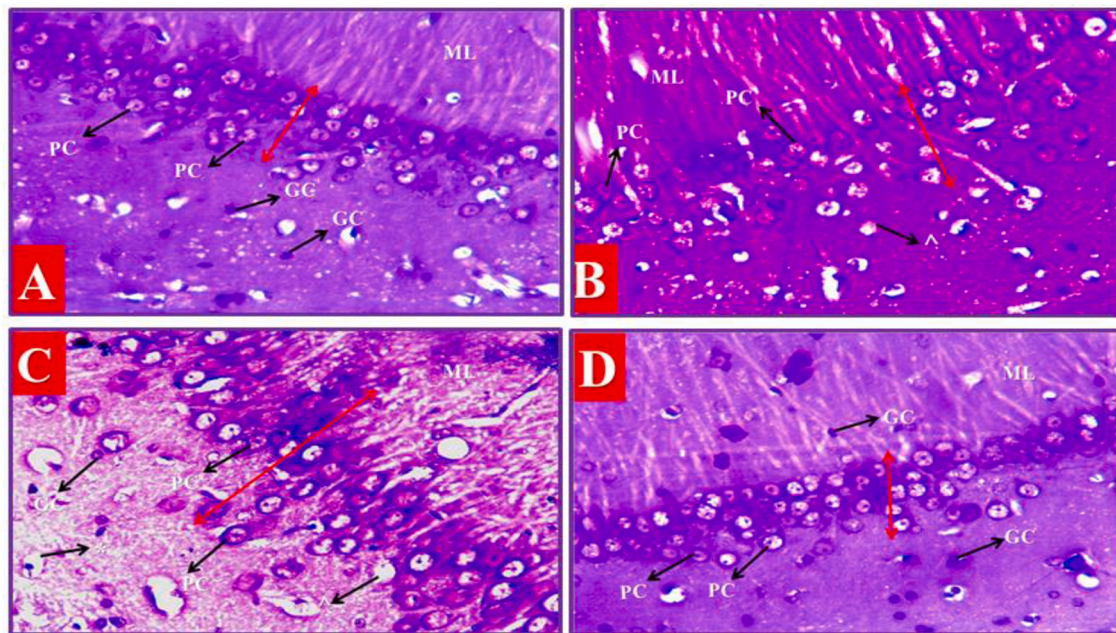


Fig. 5. Photomicrographs of sections showing the nissl bodies of neurons in Cornu Ammonis 1 in hippocampus of the brains stained with Cresyl Fast Violet [X400]. ML (Molecular Layer); PC (Pyramidal Cells); G (Glial cells); * (Deteriorated Glial Cells); ^ (Absence of Nissl bodies); Double head arrow: Pyramidal Layer (Polarized or depolarized). A (icv Saline); B (Z. officinale 300 mg/kg); C (icv STZ 3 mg/kg); D (icv STZ 3 mg/kg + Z. officinale 300 mg/kg).

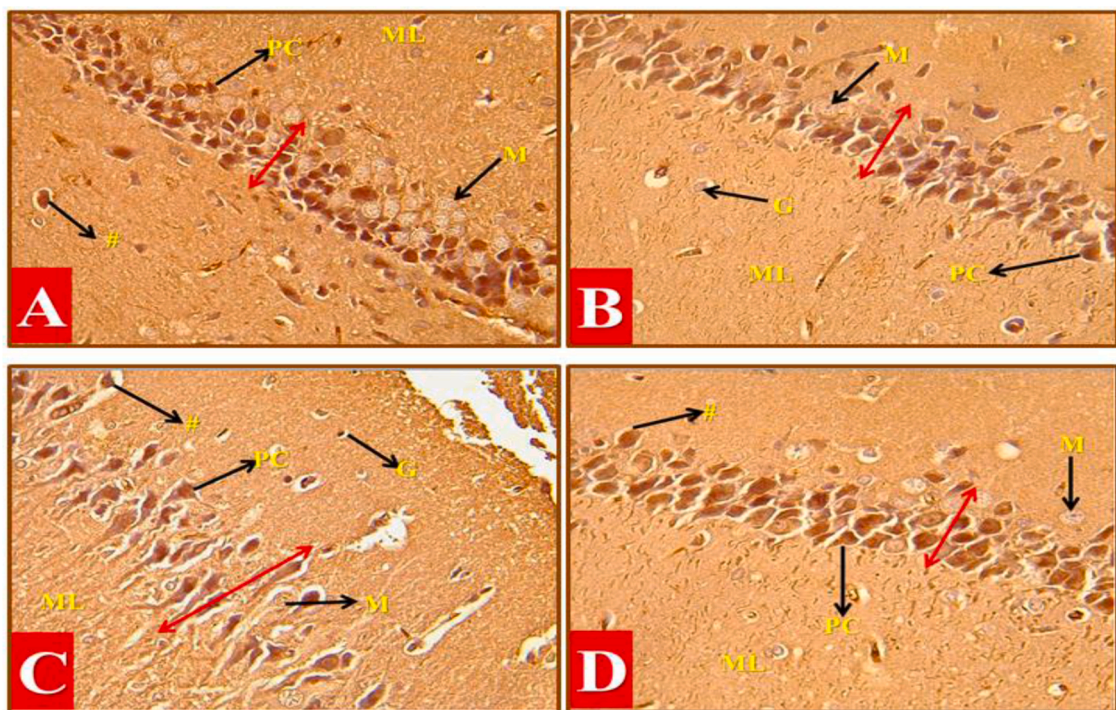


Fig. 6. Photomicrographs of sections showing the expression of Amyloid beta ($A\beta$) within the Cornu Ammonis 1 in hippocampus of the brains $A\beta$ [x400]. ML: Molecular Layer, PC: Pyramidal Cells, M: Macrophage (Microglia), G: Glial cells, #: Amyloid Beta plaques, Double head arrow: Pyramidal Layer (Polarized or depolarized). A (icv Saline); B (Z. officinale 300 mg/kg); C (icv STZ 3 mg/kg); D (icv STZ 3 mg/kg + Z. officinale 300 mg/kg).

significant reduction and increases in the OAE and CAD of rats respectively following ICV STZ treatment when compared across experimental groups. Also, SA activity of rats following ICV STZ treatment exhibited an obvious decrease, though not significant when compared with control. In furtherance, the behavioural test results reported in this study exhibits characteristic features that are observed when the functionality of the hippocampus is altered. These results are consistent with reports

from other literature (Halawany et al., 2017; Mirshekar et al., 2011; Sharma et al., 2010; Yuan et al., 2019). Treatment with Z. Officinale effectively reversed the memory loss caused by ICV STZ-induced brain insulin resistance. The possible underlying mechanisms of Z. Officinale in normalizing the neurobehavioural deficit owing to ICV STZ hyper-perfusion might be due to suggest its high level of antioxidant properties which has documented in several reports (Fouda and Berika,

Table 1
showing animal grouping.

Group (N = 8)	Administration	Dosage	Duration
A	Control	Single icv of normal saline	1
B	Z. officinale	300 mg/kg	21
C	icv STZ	3 mg/kg	1
D	icv STZ + Z. officinale	3 mg/kg + 300 mg/kg	21

Table 2
showing results for OAE and CAD of EPM and SA of Y-maze test.

Groups	OAE	CAD	SA
Control	3.600 ± 0.5099	110.4 ± 3.829	68.04 ± 17.61
ZO	3.800 ± 0.5831	93.40 ± 6.439	82.50 ± 5.000
icv of STZ	0.600 ± 0.0169 ^{ab}	176.8 ± 12.88 ^{ab}	60.22 ± 16.02 ^β
icv of STZ + ZO	3.200 ± 0.2000 ^μ	141.2 ± 2.354 ^{βθ}	77.82 ± 6.540

Control (group-A); ZO (group-B); icv of STZ (group-C); icv of STZ + ZO (group-D). NOTE: ZO = Z. *Officinale*; STZ = Streptozotocin; OAE = open arm entry; CAD = closed arm duration; SA = spontaneous alternation. α = significantly different from Control; β = significantly different from ZO; μ = significantly different from icv of STZ; θ = significantly different from icv of STZ + ZO (p ≤ 0.05).

2009; Halawany et al., 2017; Mbaveng and Kuete, 2017; Shahrajabian et al., 2019).

To further substantiate the ameliorative role of Z. *Officinale* against ICV STZ-induced brain insulin resistance, we checked for genes (relative gene expression of insulin receptor substrate (IRS), GSK 3β and Beta-secretase 1 (BACE-1)) involved in glycogen synthase kinase 3 beta (GSK3-β) signaling pathogenesis leading to neurodegeneration. GSK3-β and genes involved in its pathways are actively involved in cellular metabolism, development of neurons, and body pattern formation and has become the focus in the pathogenesis of plethora of diseases. Literature findings have reported the role of GSK3-β in neuro-development as well as in physiological and pathological aging, and its role metabolism especially in the brain (P. Salcedo-Tello et al., 2011; S. A. Martin et al., 2018; T. Jaworski et al., 2019). In our present study, a single dose of ICV STZ at 3 mg/kg resulted in a notable down-regulation of IRS in the hippocampal homogenate. Also, the relative gene expression of GSK3-β and BACE-1 in the hippocampal homogenate was significantly up regulated following a single dose of ICV STZ at 3 mg/kg. The upregulation of GSK3-β and BACE-1 genes has been directly linked to the generation of amyloid beta generation and tau protein hyper phosphorylation which is the hallmark of AD diseases. Furthermore, BACE-1 gene up-regulation has been shown to alter synaptic plasticity in rat brain (Das and Yan, 2017; Llorens-Marín et al., 2014; Phiel et al., 2003). The results of this present study are consistent with various researches that have reported the upregulation of GSK3-β and BACE-1 gene in both diabetic and AD patient as well as in brains of experimental animals (P. Salcedo-Tello et al., 2011; S.A. Martin et al., 2018; T. Jaworski et al., 2019). Contrastingly, interventions with Z. *officinale* extract in the pathogenic model attenuated this deficit as IR expression was upregulated while GSK3-β and BACE-1 gene expression was downregulated (see Fig. 3a-c). Inhibition of GSK3-β protein has been reported to deplete BACE-1-mediated cleavage of APP, Nrg1, Sez6 and Jag1 through a NF-κB signaling-mediated mechanism which is usually influenced by the generation of free radicals (Llorens-Marín et al., 2014). Thus, the possible mechanisms of action Z. *officinale* in down-regulating both GSK3-β and BACE-1 as well as upregulation of IR gene expression might be through the inhibition of NF-κB due to its antioxidant and anti-inflammatory properties which can directly influenced NF-κB signaling-mediated mechanism.

Histopathological studies have been increasingly used to identify specific mechanism that mediates behavioural changes in neurodegenerative diseases (NDDs) and this provides a good platform for seeking effective therapeutic intervention (Cummings and Zhong, 2006). Thus,

in this study histological and histochemical changes of the hippocampus were investigated using H&E and CFV staining to specifically examine the organization and arrangement of pyramidal neurons present in the CA-1 region of the hippocampus. Anti-rat amyloid β immunostaining was used to probe neurons for amyloid plaques formation. Histological presentations show that 3 mg/kg of ICV-STZ perfusion results to dispersion in the arrangement of pyramidal neurons and degenerating features such as loss of pyramidal neuronal projection which may be linked to motor and cognitive deficits, seen in the neurobehavioral test in this current study (Fig. 4). Furthermore, investigation of Nissl substances integrity in the brain of rats perfused with 3 mg/kg of ICV STZ reveals chromatolysed Nissl substance, with lightly pigmented granules which may suggest protein synthesis deficit due to ER stress. The findings of this present study is agreement with previous study that reported degenerative effects of STZ on the Nissl bodies (Vassar and Cole, 2007). This alteration may be due to trauma to the protein macromolecules and induced endoplasmic reticular stress of the neurons resulting in chromatolysis, and progressive loss of cytoarchitecture and function. While treatment with Z. *officinale* following ICV STZ perfusion significantly improve the histoarchitecture and morphological structures in the CA1 region as the cellular layer are well define and arranged with normal pyramidal neurons and axonal projections. Furthermore, Z. *officinale* treatment significantly improve the pigmentation of the Nissl granules as the Nissl bodies were highly chromatogenic. The possible mechanism action Z. *officinale* in improving the damages in the morphology of pyramidal neurons might be due to its ability to prevent ER stress and thus normalizing protein synthesis owing to it high level of antioxidant. Amyloid plaques formation is one of the major hallmark dementia and associated diseases which leads to neuron degeneration and cognitive deficit (Ghasemi Moravej et al., 2016). The result in this study revealed intense reactivity to anti-rat amyloid β with aggregation of amyloid plaques following a single dose of ICV STZ at 3 mg/kg (Fig. 6), which may be as a result of upregulation of GSK3-β and BACE-1 genes expression and correlates with the loss of neuronal cells and behavioral deficit documented in this study. Z. *officinale* treatment following STZ perfusion shows amyloid beta reactivity that is similar to that of the control groups. The ability of Z. *officinale* in reducing amyloid beta formation might be due to its ability to inhibit GSK3-β and BACE-1 activity and improve IR expression as well as inhibition of free radical generation owing to it high level of antioxidant activity.

5. Conclusion

Based on our results the generation of reactive oxygen and nitrogen species, and perturbation of protein activities in the hippocampal neurons are the possible mechanisms through which ICV STZ-induced brain insulin resistance resulting to neuronal damage. Interestingly, the treatment of rats with Z. *officinale* extract mitigated these morphological changes. Therefore, we suggest that the ameliorative mechanism shown by Z. *officinale* extract in this study may be through the inhibition of neurotoxic cascade activated by Icv STZ-induced oxidative stress, leading to experimental animal brain dysfunction.

6. The authors contribution

Olanrewaju John Afees, Bejide Ronald Ayodele, Owolabi Joshua Oladele gave the conceptual frame work for this research work. Adegbenro Gbenga carried out the research work and Olatunji Sunday Yinka, Enya Joseph Igbo, Omotoso Dayo, Arietahire Leviticus, Adelodun Stephen Taiye, Taiwo-ola Dorcas, did the write up of the article and it was proofread by Ogunbiyi Olubunmi Esther, Afolabi Toluwanimi and Fabiyi Oluwaseyi Sunday.

Conflict of Competing Interest

The authors have no conflicts of interest to declare

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Supplementary materials

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