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Original Research Article

Neuroprotective Effect of Beta-D-glucan Polysaccharide Fractionate of *Auricularia Polytrichaon* on Hyperglycaemia-Induced Cerebral Injury in Diabetic Animal Model

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ABSTRACT

An edible mushroom *Aricularia polytricha* is used by local Nigerians in managing diabetes-related complications including infertility and diabetic neuropathy but this age long practice has been going on without the corresponding acceptable experimentation. *β*-D-Glucan polysaccharide is a bioactive fractionate of *Auricularia polytricha*, an edible mushroom with nutritional and therapeutic properties. This study was intended to investigate the neuroprotective effect of *β*-D-Glucan polysaccharide on hyperglycaemia-induced cerebral injury in diabetic Wistar rats. Experiment animals were grouped into four; Group A served as normal control while groups B, C and D were induced with diabetes using 65 mg/kg.bw of streptozotocin (STZ). Diabetic animals in Groups C and D, were then treated with 120 mg/kg.bw and 200 mg/kg.bw of *β*-D-Glucan polysaccharide respectively. At termination, analysis of oxidative stress markers was done to estimate serum levels of the markers; histopathological examination was done to determine micro structural alteration of brain cells; cell quantification was also done to assess the degree of hypertrophy and proliferation of neurons. Statistical analysis was carried out using Analysis of Variance at $p<0.05$. Results showed that hyperglycaemic ambience induced a significant increase in serum level of oxidative stress markers with a concomitant increase in cell count, volume and mean size. However, levels of oxidative stress markers were significantly downgraded following *β*-D-Glucan polysaccharide administration. Glial cell aggregation and inflammatory infiltrates were also decreased when compared to diabetic control animals indicating reversal in cerebral damage. The present study suggests that *β*-D-Glucan polysaccharide has neuroprotective effect in diabetes-induced cerebral damage in Wistar rat.

Keywords: *β*-D-Glucan polysaccharide, Diabetic neuropathy, Cerebrum, Oxidative stress.

Introduction

Diabetes mellitus (DM) is one of the leading causes of mortality and morbidity among chronic diseases across the world.[1] In Sub-Saharan Africa, it is estimated that among 20 million people living with diabetes, about 62% are not diagnosed and the number is expected to reach 41.1 million by 2035.[2] Nigeria had a highest number of people with diabetes in sub-saharan Africa, with an estimated 4.7 million people of the adult population aged 20-79.[3]

A good number of diabetes – related outcomes such as generation of oxidative stress and lipid peroxidation will negatively affect individuals with both type 1 and type 2 diabetes Mellitus.[1] Numerous biochemical pathways are being triggered by hyperglycaemic ambience resulting in cerebrovascular insult. Hyperglycaemia can cause increased levels of Reactive Oxygen Species (ROS) that is capable of resulting in cellular dysfunction and mutations.[4]

Oxidative stress has been indirectly connected to the clinical consequences of microvascular and macrovascular injury.[5]Oxidative DNA damage can occur through either oxidation of DNA bases primarily by direct attack on the purine and pyrimidine bases or through strand breaks and cross-linking in DNA.[6] In a study by Maker *et al*.,[7] experimentally induced oxidative stress in-vitro significantly resulted in an increase in fragmentation, modification in base structure, deletions, clustering and frame shifts in chromatin.

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Furthermore, mitochondrial exposure to ROS provokes apoptotic process through release of Apoptosis Inducing Factor (AIF) resulting in apoptosis. This can further aggravate fragmentation in DNA strand.[8] Reactive Oxygen Species (ROS) is required in little amount for maintenance of homeostasis. However, excessive generation will overrun the internal antioxidant defense system thereby causing various degrees of damage.[8] Management and treatment of diabetes-related complications such as neuropathy have been of great concern especially in developing countries where availability, cost implication and danger of adulteration associated with drugs pose a great challenge. *β*-D-glucan polysaccharide is a fractionate of *Auricularia polytricha*, an edible mushroom known for its nutritive and therapeutic properties[9]and is used by local Nigerian communities in management of diabetes-related complications without the required scientific and clinical proof of efficacy.

Additionally,β-D-glucan polysaccharide has been found to be a good exogenous source of antioxidant as they always exist as conjugates with other biomolecules such as amino acid, protein, lipid, and nucleic acid residue.[10]As the gradual shift to herbal therapy with its attendant increasing acceptance, even among the elite confirm the claim that herbal remedies can provide cure for several diseases,[11] this study is therefore intended to investigate the neuroprotective effect of β-D-glucan polysaccharide fractionate of *Auricularia polytricha*on hyperglycaemia-induced cerebral injury in diabetic Wistar rat model.

Materials and Methods

Preparation of extract

Auricularia polytricha was obtained from a central market located in Etung Local Government Area of Cross River State on 4th day of September 2021. Authentication was done in the Department of Biological Sciences, University of Nigeria assigned voucher number 21/BS271. The fungi were dried at room temperature for one week

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and ground to powder. 200 g of *A. polytricha* was soaked in 1000 mL of ethanol, labelled and covered for 72 hours, after which a clean filter paper (Whatman No 1) was used to filter extracts. The filtrate was evaporated to dryness at 40°C in a vacuum using a rotatory evaporator. The extract was weighed and kept at 4°C in refrigerator until further use.[8]

Fractionation of β-D-glucan polysaccharide
β-D-glucan polysaccharide was experimentally separated from *A. polytricha* using acetyl trimethyl ammonium bromide to form a precipitated complex with the acidic polysaccharide. It was further purified and structurally fractionated through the use of a combination of fractional precipitation with acetic acid using ion-exchange chromatography.[12]

Experimental animals
Twenty-eight (28) adult male Wistar rats (six months old) with weight range of 150-220 g were used for this research. The rats were divided into four groups and kept in four clean cages designated A, B, C and D with seven rats in each group. The rats were allowed to acclimatize for two weeks in animal house, Department of Anatomy, Faculty of Medicine, University of Nigeria, Enugu Campus and allowed unrestricted access to commercially available chow (livestock feed) and water.
Ethical clearance number EC/FBMS/21/033 obtained from the Ethical Committee, Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus, Nigeria was assigned for this research.

Experimental design
The experimental grouping, treatment and dosage is as shown in Table 1

Induction of hyperglycaemia
After fasting for twelve hours, hyperglycaemia was induced by administering a single dose of streptozotocin (STZ) intra-peritoneally, reconstituted in 0.5M Sodium citrate and administered at a dose of 65mg/kg.bw[13]

Confirmation of diabetes
Diabetes was confirmed three days after administration of STZ using Accu-Check glucometer (Roche diagnostic, Germany) with blood samples obtained from tails of Wistar rats. Blood glucose levels at 80-120 mg/dL was considered normal while animals with hyperglycaemic levels above 120 mg/dL were considered diabetic.[14,15]

Administration of extract
Diabetic animals in Groups C and D, were treated with 120 mg/kg.bw and 200 mg/kg.bw of *β*-D-Glucan polysaccharide respectively two weeks after induction of hyperglycaemia by oral gastric intubation and lasted for ten weeks.

Histopathological studies
At termination, the animals were anaesthetised with chloroform, sacrificed and the brain tissue collected, weighed using an electronic weighing balance (Mettler Instrument AG, Switzerland) and suspended in buffered neutral formaldehyde for further processes with conventional histological techniques. Sections were cut at 5.0 µ, stained in Heamatoxylin and Eosin (H & E) and examined under a light microscope. Image J Software was used for estimating cell count and volume.[16]

Evaluation of oxidative stress markers
Oxidative stress markers were analyzed using blood obtained by cardiac puncture. Samples were transported to the laboratory for biochemical study. Oxidative stress marker kit (Sigma-Aldrich Products, Germany) was used to demonstrate for SoperoxideDimutase (SOD), Catalase and Melondialdehyde (MDA).[8]

Statistical analysis
Data obtained from this study was recorded and analyzed using one way analysis of variance (ANOVA) with SPSS program (version 20). Post-hoc test was conducted using Fischer's Least Significant Difference (LSD) to determine statistical significance among groups. Probability level of $P < 0.05$ was considered significant.

Table 1: Experimental animals were divided into four (4) groups and treated as follows

Group	Designation	Treatment	Dose
A	Normal control	Distilled water	3 mls
B	Diabetic control	Streptozotocin (STZ)	65mg/kg.bw
C	STZ + AP (Low Dose)	STZ + *β*-D-glucan polysaccharide	120mg/kg.bw
D	STZ + AP (High Dose)	STZ + β-D-glucan polysaccharide	200mg/kg.bw

Results and Discussion

Blood glucose levels
As shown in Figure 1, blood glucose levels recorded by diabetic control group (184.11±4.5) is remarkably higher when compared to the normal control (82.32±1.7) at $p < 0.05$. However, animals in group C (147.94±3.1) and D (144.02±2.1) had glucose levels slightly lower than diabetic control animals following administration of β-D-glucan polysaccharide. Glucose concentration in groups B, C and D confirm hyperglycaemic states of the experimental animals.

Biochemical analysis
Serum levels of SOD (DC: 8.11±0.4 vs NC: 2.43±1.2), catalase (DC: 3.09±2.1 vs NC: 1.49±1.0) and melondialdehyde (DC: 2.91±3.4 vs NC: 0.87±1.4); show that all oxidative stress markers in diabetic control animals were significantly higher at $p<0.05$ when compared to normal control. However, diabetic animals treated with120mg/kgbw and 200mg/kgbw of *β*-D-glucan polysaccharide had significantly ($p<0.05$) reduced activities of SOD (Group C: 4.04±2.3; Group D: 3.19±4.0), catalase (Group C: 2.07±1.6; Group D: 1.51±4.1) and melondialdehyde (Group C: 2.23±1.3; Group D: 1.27±2.0) when compared with diabetic control (Figures 2 and 3).

Cell quantification and size
As shown in table 2, cerebral cell count (DC: 3986±3.3 vs NC: 2027±4.0), total area (DC: 80,344±2.8 vs NC: 51,621±3.3) and average area (DC: 77.88±0.2 vs NC: 26.07±2.1) in diabetic control animals increased significantly $P<0.05$ when compared to normal control (Group A). However, groups C and D (Diabetic animals placed on 120 mg/kgbw and 200mg/kgbw of *β*-D-glucan polysaccharides respectively) had reduced cell count (C: 3062±4.2; D: 2633±1.6), total area (C: 73,636±2.2; D: 65,221±5.1) and mean size (C: 22.17±2.0; D: 19.72±1.1). These values were significantly lower ($p<0.05$) when compared to diabetic control animals

Table 2: Cell quantification and size in different experimental groups

Group	Cell Count	Total Area	Average Size
A	2027	51,621	17.88
B	3986*	80,344*	26.07*
C	3062*	73,636*	22.17*
D	2633*@	65,221*@	19.72*@

Values are expressed in Mean ± SEM. N = 5. ***** = Values are significantly decreased when compared to Normal Control at $p < 0.05$. **@** = Values are significantly increased when compared to Diabetic Control at $p < 0.05$.

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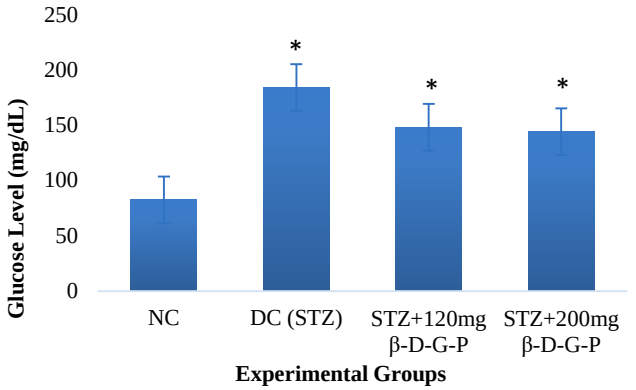

Figure 1: Comparison of blood glucose levels of different experimental groups.
Values are expressed in Mean ± SEM. N = 5. * = Values are remarkably decreased when compared to Normal Control at p<0.05.
NC – Normal Control, DC – Diabetic Control, STZ – Streptozotocin, *β*-D-G-P – Beta-D-Glucan Polysaccharide

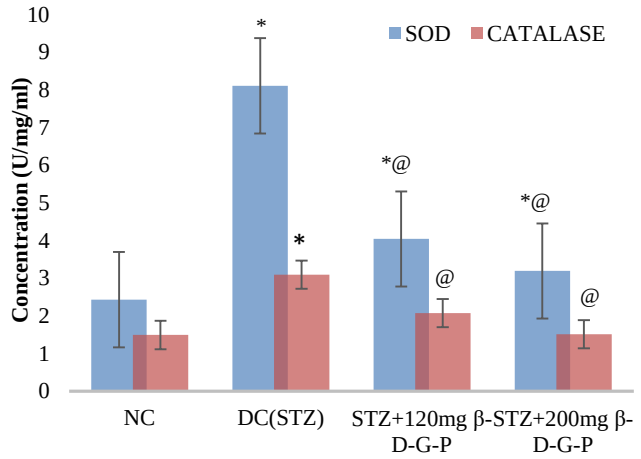

Figure 2: Comparison of Supperoxide Dimutase (SOD) and Catalase in the different experimental groups.
NC – Normal Control, DC – Diabetic Control, STZ – Streptozotocin, *β*-D-G-P – Beta-D-Glucan Polysaccharide

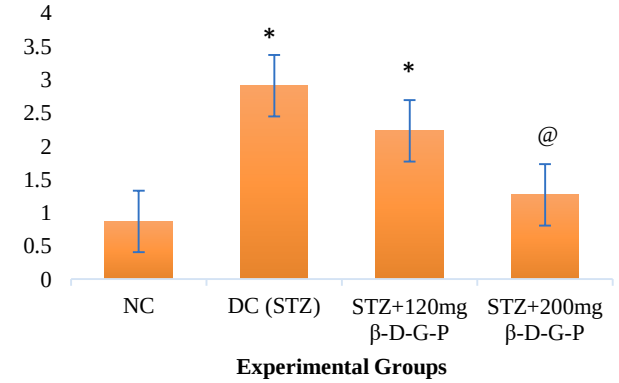

Figure 3: Comparison of Melondialdehyde in the different experimental groups.
Values are expressed in Mean ± SEM. N = 5. * = Values are significantly decreased when compared to Normal Control at p<0.05. @ = Values are significantly increased when compared to Diabetic Control at p<0.05.
NC – Normal Control, DC – Diabetic Control, STZ – Streptozotocin, β-D-G-P – Beta-D-Glucan Polysaccharide

Histological observations (Figures 4a – 4d)
Sections of histopathological sections of different experimental groups are shown in Figures 4a to 4d. Section of cerebrum in group A (Normal control) showing normal histology with no glial cells aggregation (Figure 4a). In Figure 4b, Section of cerebral cortex in group B (Diabetic control) showed cytoarchitectural alterations, the cells were mildly swollen with both intra-cytoplasm and nuclei vacuolation, aggregation of glial cell was extensive. Microglia cells were also noted with extensive inflammatory infiltrates.
Section of cerebral cortex in group C (STZ+120mg/kgbw β-D-Glucan Polysaccharide) as revealed in Figures 4c showed mild cytoarchitectural alterations, the cells were mildly swollen with aggregation of glial cell was almost absent and inflammatory infiltrates very mild. No distortions in histological sections were observed in Figure 4d (Section of cerebrum in group C placed on STZ+200mg/kgbw *β*-D-Glucan Polysaccharide). Aggregation of glial cell was absent with no inflammatory infiltrates.
This study was intended to investigate the neuroprotective effect of β-D-Glucan polysaccharide on hyperglycaemia-induced cerebral injury in a diabetic Wistar rat. Observation from this research has revealed that *β*-D-glucan polysaccharide improved antioxidant capacity and cerebral function in hyperglycaemia-induced cerebral injury. From the observation, oxidative stress markers which were significantly increased in the diabetic control were lowered slightly following β-D-glucan polysaccharide treatment. Peptide moiety in polysaccharide has been found to be responsible for free radical scavenging activity of β-D-glucan polysaccharide and lipid peroxidation inhibitory effect on superoxide and hydroxyl radicals.[17] More so, polysaccharide-protein complexes are linked to amino acids such as lysine, tyrosine, methionine, histidine and tryptophan which are capable of donating a proton to the electron-deficient reactive oxygen species (ROS).[17]
Even though a comprehensive investigation into the mechanism of action of *β*-D-Glucan polysaccharide is still lacking, it has been documented that its low molecular weight and existence in conjugate forms may trigger an interaction with certain receptors that may result in some specific therapeutic signaling pathway to benefit host organ (cerebrum). Furthermore, β-D-Glucan polysaccharide are found to possess lipid peroxidation inhibitory effect reasons being that polysaccharide-polyphenol conjugates are known to be mediated by hydrophobic interactions in the cell membranes as a consequence, hydrophobic cavities and crevasses may exist for these conjugates thereby preventing damage of the cell membrane. Renard *et al*.[18] reported similar findings.
This study has also demonstrated that brain damage in the diabetic control group (DC) is evident in aggregation of glial cells, presence of inflammatory infiltrates and severe neuronal loss in the cerebral cortex. Very critical histopathological activities have been reported in the brain during hyperglycaemic injury given to the glucose utilization. Cerebral inflammation and its vascular complications have been documented in diabetes-induced brain injury.[19] Aggregation of glial cell is a first line biomarker indicating neural damage and are activated by inflammatory pathways and cytotoxic product such as ROS and interleukin. Consequently, glial cells are activated in response to brain injury and increase in aggregation of these cells is a consequence of the severity of inflammation.[19]
A significantly higher cell count, sizes and volume of cerebral cells of diabetic control animals when compared to normal control is indicative of brain damage. This is consistent with findings from Selim and Selim (2013)[20] who reported that hypertrophy and proliferation of neurons is in response to chemical and mechanical insult meant to enhance structural and functional changes occasioned by hyperglycaemia-induced cerebral injury. This forms the basis for pathogenesis of neurodegenerative diseases. However, animals in groups C and D (diabetic animals placed on 120 mg/kgbw and 200 mg/kgbw of *β*-D-glucan polysaccharides respectively) showed progressively reduced cell count, volume and mean size with lower degree of glial cells aggregation and reduced inflammatory infiltrates accompanied by neuronal loss indicating reversal in cerebral damage.

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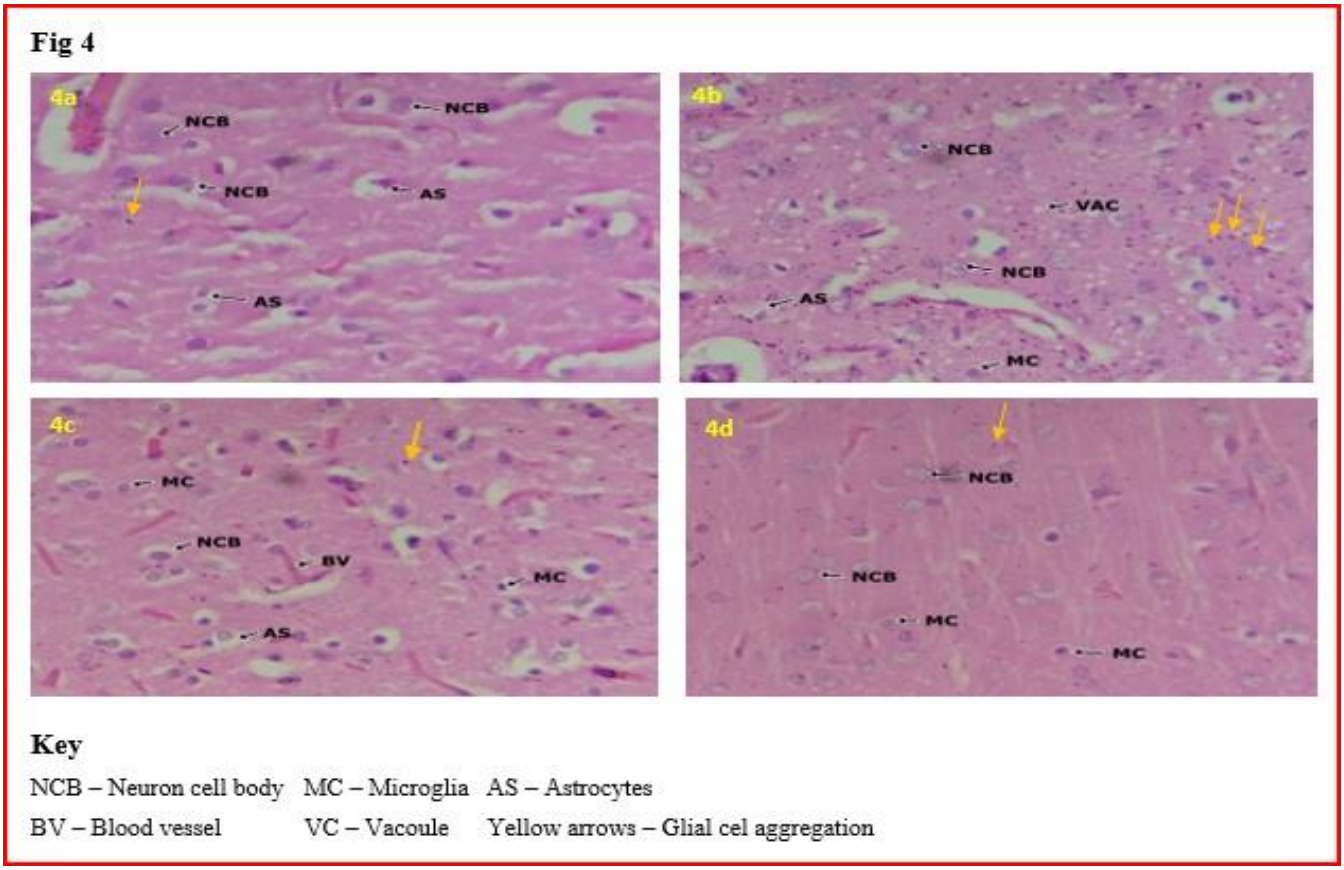

Figure 4: (a) Section of cerebrum in group A (Normal control) showing normal histology with no glial cell aggregation. **(b)** Section of cerebrum in group B (Diabetic control). Section of cerebral cortex showed alterations, the cells were mildly swollen with both intra-cytoplasm and nuclei vacuolation, aggregation of glial cell was extensive. Microglia cells were also noted with extensive inflammatory infiltrates. **(c)** Section of cerebrum in group C (STZ+120mg/kgbw β-D-Glucan Polysaccharide). Section of cerebral cortex showed mild alterations, the cells were mildly swollen with aggregation of glial cell was almost absent and inflammatory infiltrates very mild. **(d)** Section of cerebrum in group C (STZ+200mg/kgbw β-D-Glucan Polysaccharide). Section of cerebral cortex showed no alterations. Aggregation of glial cell was absent with no inflammatory infiltrates.

Conclusion

β-D-glucan polysaccharide has been shown from this research to regulate diabetes-induced oxidative stress generation, suppress glial cells aggregation and decrease cell count, sizes and volume in cerebrum of hyperglycaemic rat. The underlying mechanism of this neuroprotective effect can be traced to its strong antioxidant capacity. This study may have suggested that *β*-D-glucan polysaccharide fractionized from edible fungus *A. polytricha*is a potential antioxidant, anti-inflammatory and neuroprotective agent capable of ameliorating diabetes-induced cerebral injury.

Conflict of Interest

The authors declare no conflict of interest**.**

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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