

Neuroprotective effect of beta-D-glucan polysaccharide on hyperglycaemia-induced cerebral injury in diabetic animal model

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SUMMARY

An edible mushroom, *Auricularia 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 clinical trial and acceptable experimentation. β -D-Glucan polysaccharide is a bioactive fractionate of *Auricularia polytricha*, an edible mushroom with nutritional and therapeutic property. This study was intended to investigate the neuroprotective effect of β -D-Glucan polysaccharide on hyperglycaemia-induced cerebral injury in diabetic Wistar rats. Experimental animals were grouped into four: Group A served as normal control and was placed on distilled water, 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 treated with 120 mg/kg bw and 200 mg/kg bw of β -D-Glucan polysaccharide respectively. Group B served as diabetic control animals. An analysis of oxidative stress markers (superoxide dismutase, catalase and melondialdehyde) 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. Increase in glial cells aggregation in the cerebral cortex are indicators of cerebral damage in diabetic control animals. However, levels of oxidative stress markers were significantly downgraded following β -D-Glucan polysaccharide administration. Glial cell aggregation and inflammatory infiltrates were also decreased in diabetic models placed on β -D-Glucan polysaccharide 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.

Key words: *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

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diseases across the world (Bhatia et al., 2010). In Sub-Saharan Africa, it is estimated that among 20 million people are living with diabetes, about 62% are not diagnosed and the number is expected to reach 41.1 million by 2035 (Dahiru et al., 2016). 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 (Ugwu et al., 2020).

A good number of diabetic-related outcomes such as generation of oxidative stress and lipid peroxidation will negatively affect individuals with both type 1 and type 2 diabetes Mellitus (Bhatia et al., 2010). Numerous biochemical pathways are being triggered by high hyperglycemic ambience resulting in cerebrovascular insult. Hyperglycemia is also capable of increasing levels of Reactive Oxygen Species (ROS), thereby resulting in cellular dysfunction and mutations (Nishikawa et al., 2000).

Oxidative stress has been indirectly connected to the clinical consequences of microvascular and macrovascular injury (Baker et al., 2004). 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 (Chaborry et al., 2009). In a study by Maker et al. (2009), experimentally induced oxidative stress in vitro significantly resulted in an increase in fragmentation, modification in base structure, deletions, clustering and frame shifts in chromatin. 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 (Agbor and Anyanwu, 2020). 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 (Agbor and Anyanwu, 2020).

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 (Chang et al., 2011), 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 antioxidants, as they always exist as conjugates with other biomolecules such as amino acid, protein, lipid, and nucleic acid residue (Nie et al., 2011). 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 (Anthony et al., 2006), 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 models.

MATERIALS AND METHODS

Preparation of extract

Auricularia Polytricha was obtained from a central market located in Etung Local Government Area of Cross River State, Nigeria. It was authenticated in the Department of Biological Sciences, University of Nigeria. The fungi were dried at room temperature for one week and ground to powder. 200g of *A. polytricha* was soaked in 1000ml of ethanol, labelled and covered for 72 hours, after which a clean filter paper (Watman 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 then weighed and kept at 4°C in refrigerator until further use. (Agbor and Anyanwu, 2020).

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 through a combination of fractional precipitation with acetic acid using ion-exchange chromatography (Zhang et al., 2007).

Experimental animals

Ethical clearance for this research was obtained from the Ethical Committee, Faculty of Basic Medical Sciences, University of Calabar, Nigeria. 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 the 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.

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 streptozotocin (STZ) intra-peritoneally, reconstituted in 0.5M Sodium citrate and administered at a dose of 65 mg/kg.bw (Ugochukwu and Babady, 2003).

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 were considered normal, while animals with hyperglycaemic levels above 120 mg/dl were considered diabetic. (Anyanwu and Agbor, 2020, 2021).

Administration of extract

β -D-glucan polysaccharide administration commenced two weeks after induction of hyperglycaemia by oral gastric intubation and lasted for ten weeks.

Histopathological studies

At termination, the animals were 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 m, stained in Hematoxylin and Eosin (H&E) and examined under a light microscope. Image J Software was used for estimating cell count and volume (Oyesolape et al., 2020).

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 Soperoxide Dimutase (SOD), Catalase and Melondialdehyde (MDA) (Agbor and Anyanwu, 2020).

Statistical analysis

Data obtained from this study were 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 ml
B	Diabetic control	Streptozotocin (STZ)	65 mg/kg.bw of STZ
C	STZ + AP (Low Dose)	STZ + β -D-glucan polysaccharide	65 mg/kg.bw of STZ + 120 mg/kg.bw of β -D-glucan polysaccharide
D	STZ + AP (High Dose)	STZ + β -D-glucan polysaccharide	65 mg/kg.bw of STZ + 200 mg/kg.bw of β -D-glucan polysaccharide

RESULTS

Blood glucose levels

As shown in Fig. 1, blood glucose levels recorded by diabetic control group (184.11 ± 4.5) were 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 confirms hyperglycemic 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 with 120 mg/kg bw and 200 mg/kg bw 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 (Figs. 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: $80,344 \pm 2.8$ vs NC: $51,621 \pm 3.3$) 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/kg bw and 200 mg/kg bw of β -D-glucan polysaccharides respectively) had reduced cell count (C: 3062 ± 4.2 ; D: 2633 ± 1.6), total area (C: $73,636 \pm 2.2$; D: $65,221 \pm 5.1$) and mean size (C: 22.17 ± 2.0 ; D: 19.72 ± 1.1). These values were significantly lower (at $p < 0.05$) when compared to diabetic control animals.

Histological observations (Figs. 4a-4d)

Histopathological sections of different experimental groups are shown in Figs. 4a to 4d. Section of cerebrum in group A (Normal

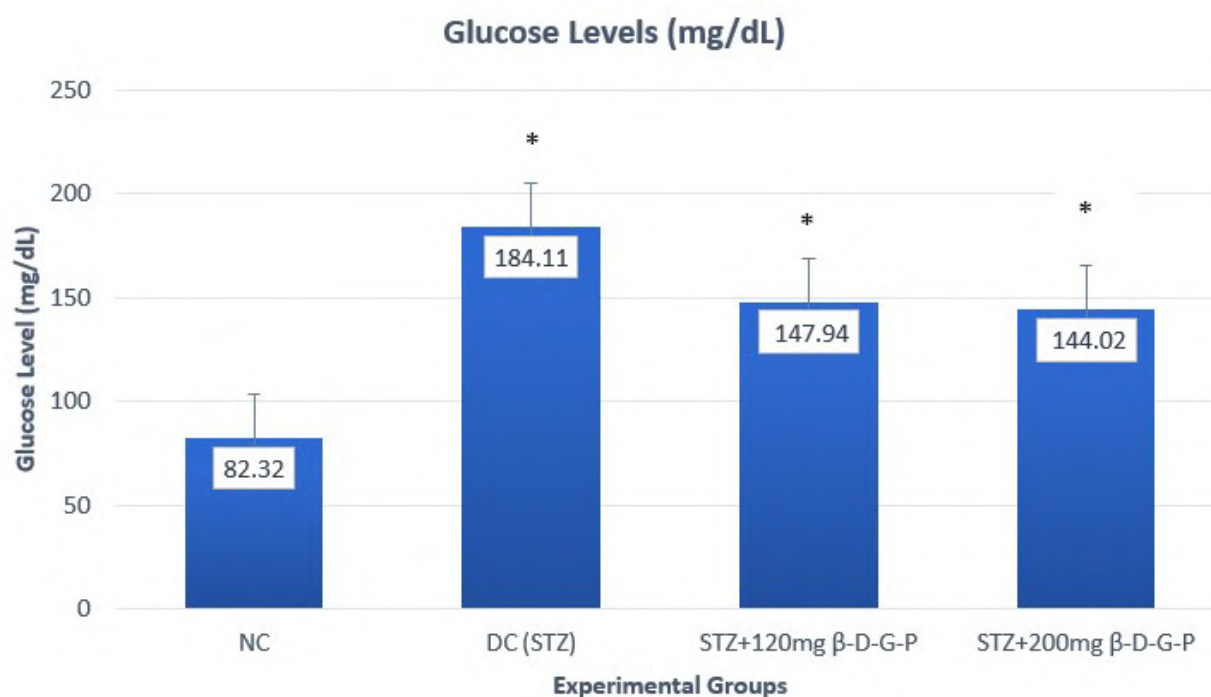


Fig 1.- Comparison of blood glucose levels of different experimental groups. Values are expressed as mean \pm SEM. N = 5. * = Values are remarkably decreased when compared to normal control at $p < 0.05$. NC – Normal Control, DC – Diabetic Control, STZ – Streptozotocin, β -D-G-P – Beta-D-Glucan Polysaccharide.

control) showed normal histology with no glial cells aggregation (Fig. 4a). In Fig. 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.

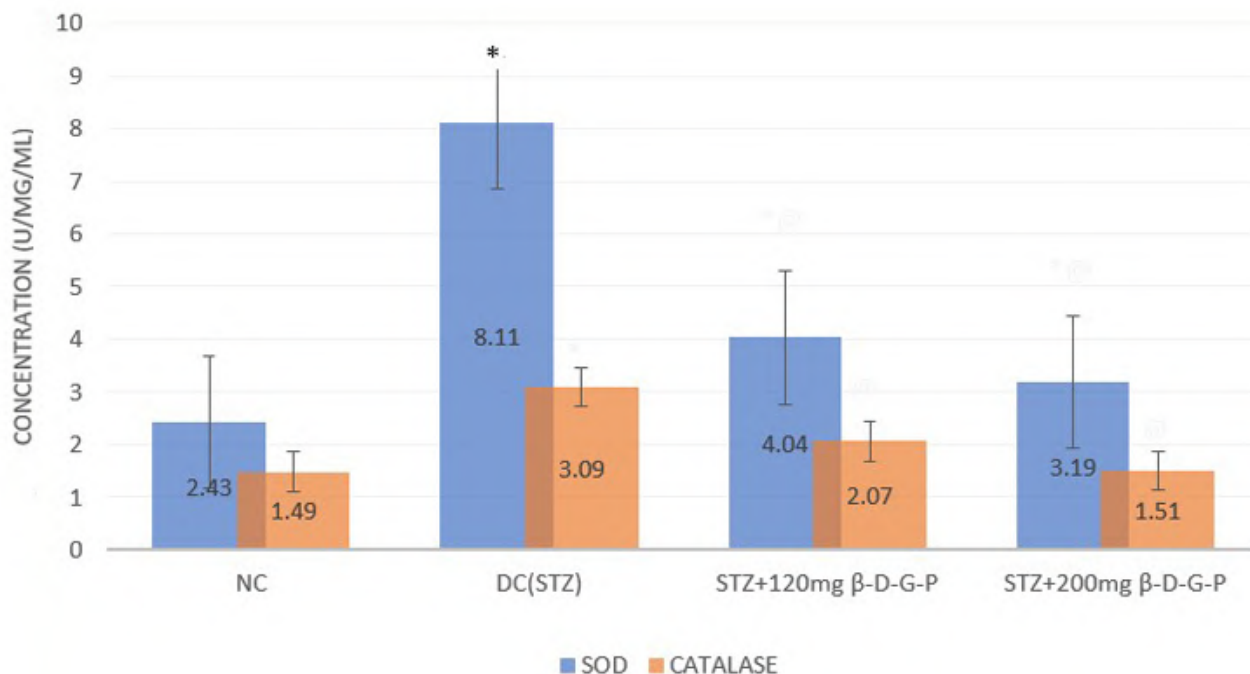
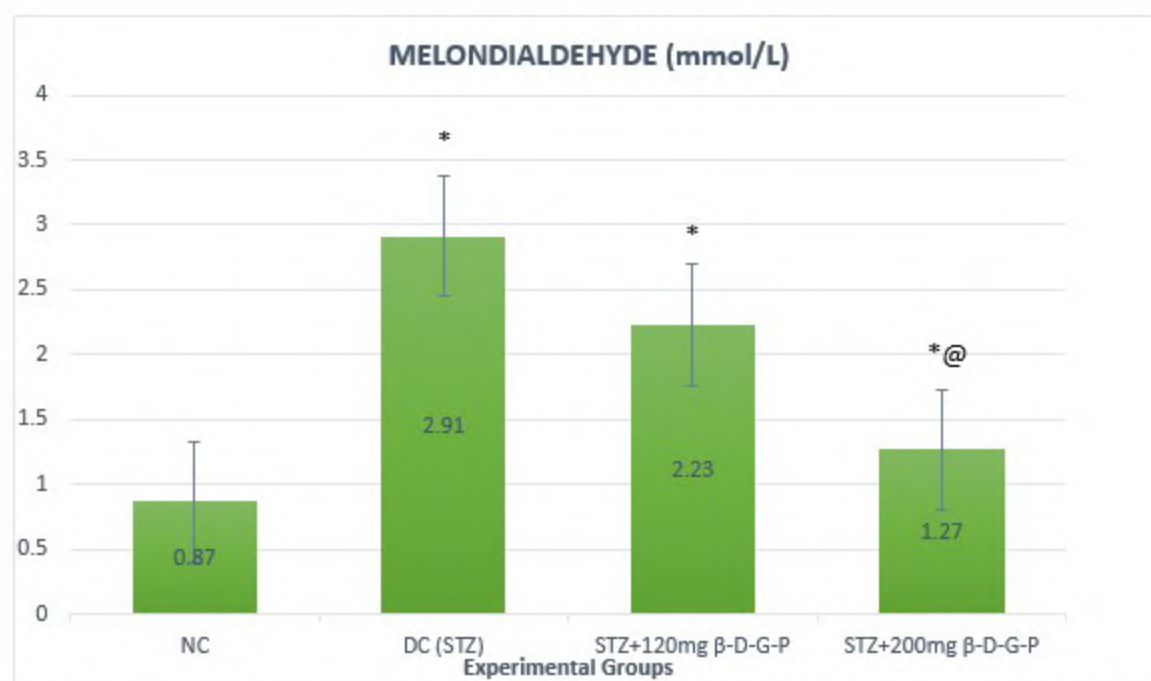


Fig. 2.- Comparison of superoxide dismutase (SOD) and catalase in the different experimental groups. Values are expressed as mean \pm SEM. N = 5. * = Values are significantly decreased when compared to normal control at $p < 0.05$. @ = Values are significantly increased when compared to diabetic control at $p < 0.05$. NC – Normal Control, DC – Diabetic Control, STZ – Streptozotocin, β -D-G-P – Beta-D-Glucan Polysaccharide.



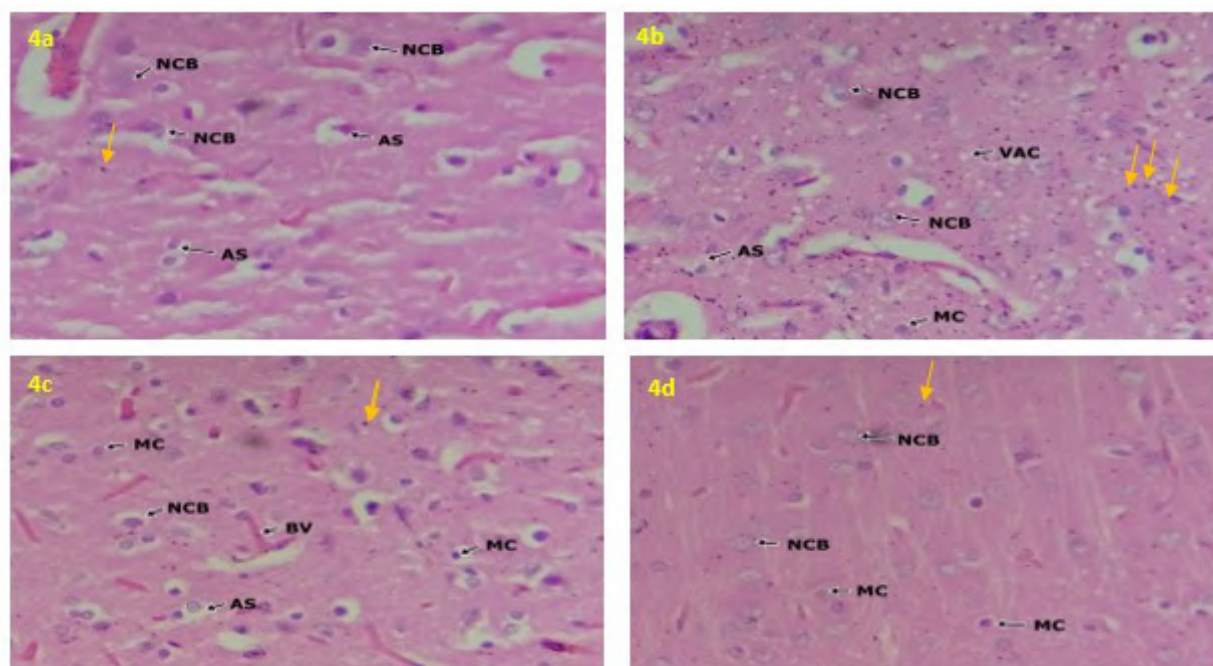
NC – Normal Control, DC – Diabetic Control, STZ – Streptozotocin, β -D-G-P – Beta-D-Glucan Polysaccharide

Fig. 3.- Comparison of melondialdehyde in the different experimental groups. Values are expressed as Mean \pm 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 $p < 0.05$.

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 as mean \pm SEM. N = 7. * = Values are significantly increased when compared to Normal Control at $p < 0.05$. @ = Values are significantly decreased when compared to Diabetic Control at $p < 0.05$.



Key

NCB – Neuron cell body MC – Microglia AS – Astrocytes
 BV – Blood vessel VC – Vacuole Yellow arrows – Glial cell aggregation

Fig. 4.- Sections of the cerebral cortex in all groups studied. **4a:** Section of cerebrum in group A (Normal control) showing normal histology with no glial cell aggregation. **4b:** Section of cerebral cortex in group B (Diabetic control) showing alterations, the cells were mildly swollen with both intracytoplasmic and nuclei vacuolation, aggregation of glial cells was extensive. Microglial cells were also noted with extensive inflammatory infiltrates. **4c:** Section of cerebral cortex in group C (STZ+120 mg/kg bw β -D-Glucan polysaccharide) showing mild alterations, the cells were mildly swollen with aggregation of glial cell was almost absent and inflammatory infiltrates very mild. **4d:** Section of cerebral cortex in group C (STZ+200 mg/kg bw β -D-Glucan polysaccharide) showing no alterations. Aggregation of glial cell was absent with no inflammatory infiltrates. NCB – neuron cell body; MC – Microglia; AS – Astrocytes; BV – Blood vessel; VC – Vacuole; Yellow arrows – Glial cell aggregation.

Section of cerebral cortex in group C (STZ + 120 mg/kg bw β -D-Glucan polysaccharide) as revealed in Fig. 4c showed mild cytoarchitectural alterations; the cells were mildly swollen, aggregation of glial cell was almost absent and inflammatory infiltrates were very mild. No distortions in histological sections were observed in Fig. 4d (section of cerebrum in group C placed on STZ+200 mg/kg bw β -D-Glucan polysaccharide). Aggregation of glial cell was absent with no inflammatory infiltrates.

DISCUSSION

This study was intended to investigate the neuroprotective effect of β -D-Glucan polysaccharide on hyperglycaemia-induced cerebral injury in diabetic Wistar rats. Observation from this research has revealed that β -D-glucan polysaccharide improved antioxidant capacity and cerebral function in hyperglycemia-induced cerebral injury. From the observation, oxidative stress markers, which significantly increased in the diabetic control, were lowered slightly following β -D-glu-

can 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 (Lee et al., 2003). 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). 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 is found to possess lipid peroxidation inhibitory effect, the 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. (2001) 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 hyperglycemic injury given to the glucose utilization. Cerebral inflammation and its vascular complications have been documented in diabetes-induced brain injury (Drake et al., 2011). Aggregation of glial cells is a first line biomarker indicating neural damage, and is 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 (Drake et al., 2011).

A significantly higher cell count, sizes and volume cerebral cells of diabetic control animals when compared to normal control are indicative of brain damage. This is consistent with findings

from Selim and Selim (2013), 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 hyperglycemia-induced cerebral injury. This forms the basis for pathogenesis of neurodegenerative diseases. However, groups C and D, diabetic animals placed on 120 mg/kg bw and 200mg/kg bw of β -D-glucan polysaccharides respectively, had progressively reduced cell count, volume and mean size accompanied by lower degree of glial cell aggregation and reduced inflammatory infiltrates accompanied by neuronal loss indicating reversal in cerebral damage.

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 hyperglycemic 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.

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AUTHORS' CONTRIBUTIONS

All authors gave equal contribution to this research.

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