

Antioxidant effect of Beta-D-glucan-polysaccharide fractionate of *Auricularia polytricha* on Hyperglycaemia-Induced Kidney Dysfunction in Experimental Diabetic Nephropathy

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Abstract

β -D-Glucan polysaccharide is a major bioactive component fractionated from *Auricularia polytricha* (mushroom), an edible fungus used for both nutritional and therapeutic purposes. Local Nigerian men use *A. polytricha* to manage diabetes related complications without accepted experimentation to ascertain the role and mechanism of action of its fractionates. This study was intended to find out the effect of β -D-Glucan polysaccharide supplementation of hyperglycaemia-induced kidney dysfunction in diabetic Wistar rat. Experiment animals were divided into four groups and received treatment as follows; Group A (treated with normal saline and served as normal control), Group B, (diabetic control animals were treated with 65mg/kg.bw of streptozotocin (STZ)), Groups C and D, (placed on 120 mg/kg.bw and 200 mg/kg.bw of β -D-Glucan polysaccharide respectively after inducing diabetics). At termination, 24 hours urine was collected and analyzed for creatinine clearance and proteinuria, both kidneys were harvested and weighed, serum was collected by cardiac puncture and analysed for serum creatinine and oxidative stress markers (Superoxide Dismutase, Catalase and Malondialdehyde), while histopathological examination was done using routine Hematoxyline and Eosine technique with the help of light microscope. The result showed that hyperglycaemic ambience caused a significant increase in serum level of oxidative stress markers along with concomitant cytoarchitectural alterations of kidney tissue and a change in values of renal parameters (serum creatinine, proteinuria, and creatinine clearance) indicating kidney dysfunction in diabetic control animals. However, decrease in activities of oxidative stress markers, reversal of alterations in kidney microstructure, and the overall improvement in values of renal parameters were observed following administration of β -D-Glucan polysaccharide in a dose-dependent manner. The present study suggests that β -D-Glucan polysaccharide supplementation can ameliorate hyperglycaemia-induced nephrotoxicity in diabetic wistar rat model.

Keywords: β -D-Glucan polysaccharide, diabetic nephropathy, *Auricularia polytricha*, kidney, oxidative stress

1. Introduction

Diabetic nephropathy is one of the major complications associated with diabetes mellitus. It is characterized by accumulation of extracellular matrix in the glomerular and tubulointerstitial compartments of the kidney which may result in renal failure (Nangaku et al. 2004). Researchers have described cellular events during diabetic nephropathy to include generation of Reactive Oxygen Species (ROS), channeling of glucose intermediates into metabolic pathways resulting in generation of advanced glycation product and the increase in expression of transforming growth factor. The result is hyperglycaemia-induced injury from excessive generation of ROS thereby causing an alteration in intraglomerular hemodynamics (Singh et al. 2008).

Both glomerular and tubulointerstitial injuries are involved in pathogenetic mechanisms of diabetic nephropathy. Diabetic kidney injury begins with hyperglycaemia which in turn creates a redox environment in the vasculature, nephron and surrounding interstitium

causing dysfunction of virtually all types of kidney cells (Phillips et al. 2003). Tubulointerstitial injury develops after glomerular injury and correlates with alteration in renal parameters such as decreased Glomerular Filtration Rate (GFR), increase in proteinuria and decrease in creatinine clearance (Nangaku et al. 2004).

Oxidative stress caused by increased levels of ROS serve as an inducer and amplifier of signaling cellular events that occur in high glucose ambience (Ha and Lee, 2005; Brownlee, 1995). ROS is required in small amounts necessary to maintain cellular homeostasis, but sustained hyperglycaemia in diabetic conditions will cause a dramatic rise in ROS thereby damaging target organs (Lee et al. 2003). Mechanism of reactive oxygen species generation involves, high oxygen concentration reduced to water, while part of it is transformed to O_2^- in the mitochondria (Brand, 2010). This is capable of causing cellular dysfunction, apoptosis and mutations (Nishikawa et al., 2000)

There has been a shift to herbal therapy with an increasing acceptance in recent times, even among the elite confirming that that herbal therapy is potent in

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management and treatment of several diseases (Anthony *et al.*, 2006). Numerous animal and plant products have been reported to have medicinal properties (Bhatia *et al.*, 2010). β -D-Glucan polysaccharide is a major bioactive constituent of *Auricularia polytricha* (mushroom), an edible fungus used globally for culinary and therapeutic purposes. β -glucans are glucose polymers polysaccharides linked together by a 1-3 linear β -glycosidic chain core and are found as a major constituent in the cell wall of fungus. They differ from each other by their length and branching structures (Stone and Clarke, 1992) and do not always exist in single form but as conjugate with other biomolecules such as amino acid, protein, lipid and nucleic acid residue. More so, its low molecular weight, chelating effect and chemical modification by biochemical intermediates makes them a strong source of exogenous antioxidant (Nie *et al.*, 2011).

Local Nigerian men have used *A. polytricha* to manage complications in diabetic men without reliable experimentation to back up their claim. This study tried to find out the antioxidant effect of β -D-Glucan polysaccharide fractionate of *A. polytricha* on experimental diabetes nephropathy using Wistar rat model.

2. Materials And Methods

2.1. Collection of Mushroom

A. Polytricha was purchased from Etomi central market in Etung Local Government Area of Cross River State. It was then taken to Department of Biological Sciences, University of Nigeria for identification. The mushroom was dried under room temperature, powdered and then extracted with ethanol (Anyanwu and Agbor, 2020).

2.2. 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 described through the use of a combination of fractional precipitation with acetic acid using ion-exchange chromatography. (Zhang *et al.* 2007)

2.3. Experimental Animals

Sixteen (16) adult male Wistar rats were weighed and grouped into four in clean cages with four rats in each. The rats were kept for two weeks of acclimatization in animal house, Department of Anatomy, Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus and allowed free access to available chow and water.

2.4. Experimental Design

The experimental grouping, treatment and dosage are shown in Table 1

Table 1. Experimental animals were divided into four (4) groups with 4 animals in each. Treatment received is as follows:

Group	Number of Animals	Treatment	Dose
A	4	Distilled water	3 mls
B	4	Streptozotocin (STZ)	65mg/kg.bw
C	4	STZ + β -D-glucan polysaccharide	120mg/kg.bw
D	4	STZ + β -D-glucan polysaccharide	200mg/kg.bw

2.5. Induction of Hyperglycaemia

Diabetes was induced by administration of 65 mg/kgbw of Streptozotocin (STZ) intraperitoneally after allowing the animals to fast for 12 hours, STZ was reconstituted in 0.5M of Sodium citrate before administration (Ugochukwu and Babady 2003).

2.6. Confirmation of Hyperglycaemia

Accu-Check glucometer (Roche diagnostic, Germany) was used to confirm diabetes 3 days after STZ administration, using blood samples from the tails of experimental animals. Blood glucose levels was checked before and after induction to confirm diabetic state (Anyanwu and Agbor, 2020).

2.7. Administration of Extract

After two weeks of sustained hyperglycaemia, β -D-glucan polysaccharide was administered via oral gastric intubation. to experimental groups (B, C and D) once daily. The experiment lasted for 2 weeks,

2.8. Collection of Tissues and Samples for Analysis

At termination, kidneys were harvested weighed together using an electronic weighing balance (Mettler Instrument AG, Switzerland) and suspended in buffered neutral formaldehyde for further processes with conventional histological techniques. Paraffin sections were cut at 5.0 microns using a Manual Rotary Microtome. Hematoxylin and Eosin (H & E) stained sections were observed under a light microscope.

2.9. 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 kits (Sigma-Aldrich Products, Germany) was used to demonstrate for Superoxide Dismutase (SOD), Catalase and Malondialdehyde (MDA) (Anyanwu and Agbor, 2020).

2.10. Analysis for Renal Parameters

Urine was obtained from metabolic cages housing the experimental animals on the last day, before sacrifice. Creatinine auto-analyzer (Beckman Instrument Fullerton CA) was used to evaluate urine and serum creatinine levels while spectrophotometric assay was adopted in estimation of total protein (Sharma *et al.*, 2001).

2.11. 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.

3. Results

3.1. Weight of Kidney

As shown in Figure 1, a significantly ($p < 0.05$) lower kidney weight (0.80 ± 0.34 g) was observed in diabetic control (Group B) when compared to normal control (1.82 ± 0.67 g). However, hyperglycaemic animals that received 120 mg/kg.bw and 200mg/kg.bw of β -D-glucan polysaccharide in Groups C and D respectively recorded kidney weights that were significantly ($p < 0.05$) higher when compared to the diabetic control.

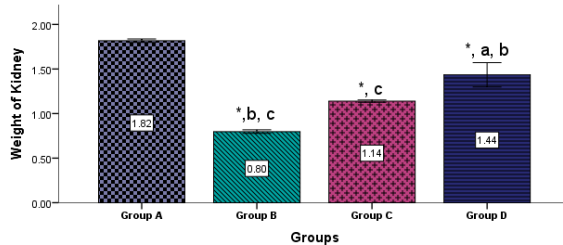


Fig 1: Comparison of Weight of Kidney in different experimental Groups
Values are expressed as mean \pm SEM, n = 4.
* = significantly different from Group A at $p < 0.05$
a = significantly different from Group B at $p < 0.05$
b = significantly different from Group C at $p < 0.05$
c = significantly different from Group D at $p < 0.05$

3.2. Biochemical Analysis

High serum SOD, Catalase and Malondialdehyde (MDA) level were recorded by the diabetic control animals and these values were significantly ($p < 0.05$) higher when compared to normal control group. However, all the diabetic animals treated with graded doses of β -D-glucan polysaccharide had significantly lower SOD, catalase and Malondialdehyde activities when compared with diabetic control (Figures 2, 3 and 4).

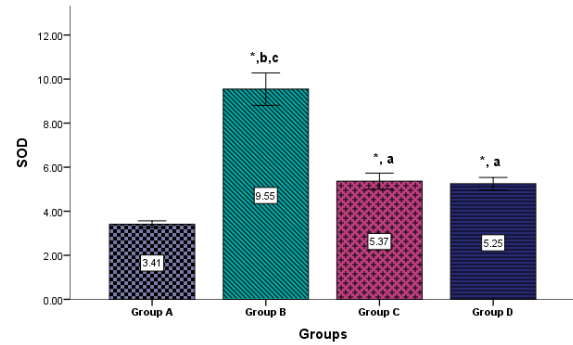


Fig 2: Comparison of SOD level in different experimental Groups
Values are expressed as mean \pm SEM, n = 4.
* = significantly different from Group A at $p < 0.05$
a = significantly different from Group B at $p < 0.05$
b = significantly different from Group C at $p < 0.05$
c = significantly different from Group D at $p < 0.05$

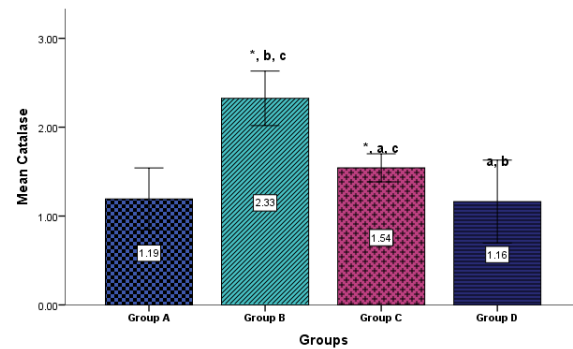


Fig 3: Comparison of Catalase level in different experimental Groups
Values are expressed as mean \pm SEM, n = 4.
* = significantly different from Group A at $p < 0.05$
a = significantly different from Group B at $p < 0.05$
b = significantly different from Group C at $p < 0.05$
c = significantly different from Group D at $p < 0.05$

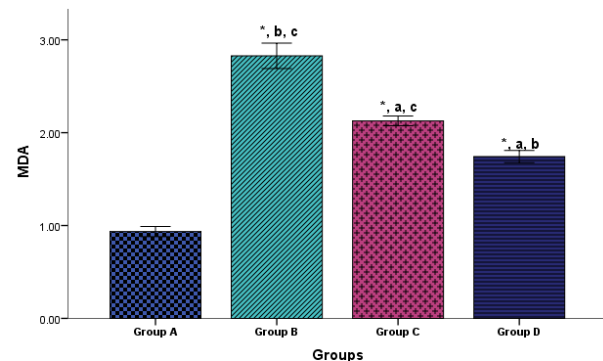
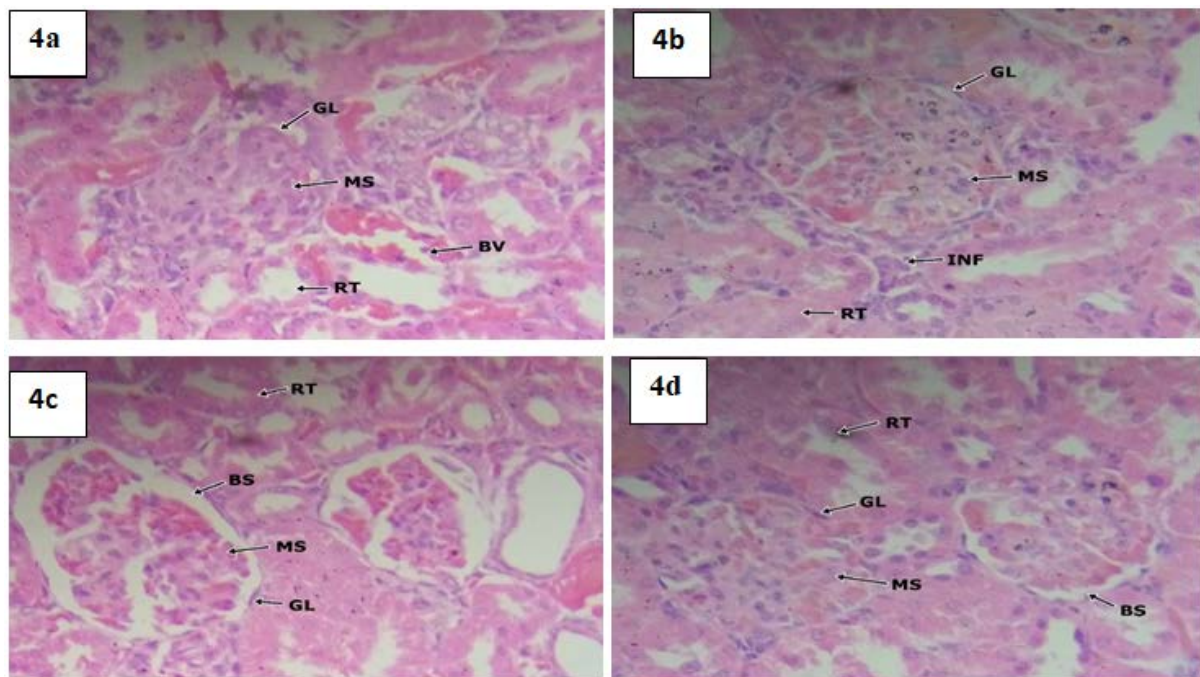


Fig 4: Comparison of MDA level in different experimental Groups
Values are expressed as mean \pm SEM, n = 4.
* = significantly different from Group A at $p < 0.05$
a = significantly different from Group B at $p < 0.05$
b = significantly different from Group C at $p < 0.05$
c = significantly different from Group D at $p < 0.05$

3.3. Histopathological Examination

Fig. 4: Histopathological Examination of Kidney – X400



Key GL – Glomerulus MS – Mesangium RT – Renal tubule BS – Bowman's Space BV – Blood Vessel.

Figure 4. a) Section of the kidney in group A (Normal control) showing prominent renal tubules and glomeruli. The intervening stroma is scanty and consists of sparse interstitial cells and dilated blood vessels. The glomeruli and Bowman's space were seen intact. b) Section of the kidney of animals in Group B (Diabetic control) showing prominent renal tubules and glomeruli with diffused expansion and proliferation of mesangium, thickening of glomerular basement membrane and renal tubular hypertrophy. The intervening stroma consists of inflammatory infiltrates. The glomeruli are mildly swollen with a distorted Bowman's space. Extensive haemorrhage is seen within the mesangial matrix. c) Section of the kidney in group C (Diabetic animals placed on 120mg/kg.bw β -D-glucan polysaccharide) shows prominent renal tubules and glomeruli. The intervening stroma is scanty and consists of sparse interstitial cells and mild inflammatory infiltrates. The glomeruli are atrophic with an intact Bowman's space and a cellular mesangium. d) Section of the kidney in group D (Diabetic animals placed on 200mg/kg.bw β -D-glucan polysaccharide) shows prominent renal tubules and glomeruli. The intervening stroma is scanty and consists of sparse interstitial cells and mild inflammatory infiltrates. The glomeruli have intact Bowman's space and a cellular mesangium.

3.4. Renal Parameters

As shown in figures 6, 7 and 8, serum creatinine level (2.58 ± 0.44 mg/dl) in diabetic control (Group B) was remarkably increased when compared to normal control (0.69 ± 0.84), proteinuria in diabetic control group was also significantly higher when compared to normal control, whereas creatinine clearance in diabetic control group was significantly ($p < 0.05$) lower when compared to normal control.

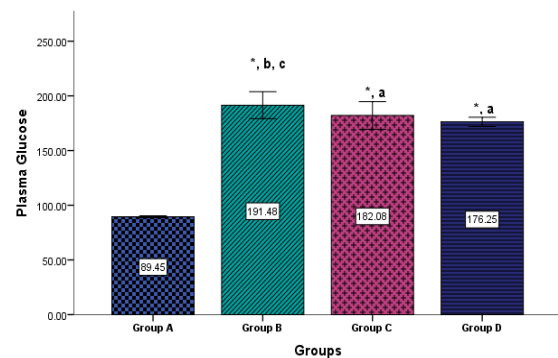


Fig 5: Comparison of Plasma glucose level in different experimental Groups
Values are expressed as mean \pm SEM, n = 4.
* = significantly different from Group A at $p < 0.05$
a = significantly different from Group B at $p < 0.05$
b = significantly different from Group C at $p < 0.05$
c = significantly different from Group D at $p < 0.05$

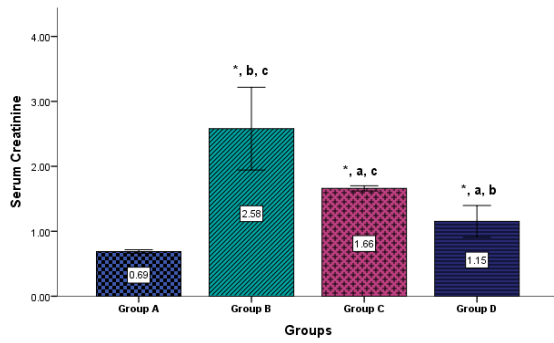


Fig 6: Comparison of Serum creatinine level in different experimental Groups
 Values are expressed as mean \pm SEM, n = 4.
 * = significantly different from Group A at $p < 0.05$
 a = significantly different from Group B at $p < 0.05$
 b = significantly different from Group C at $p < 0.05$
 c = significantly different from Group D at $p < 0.05$

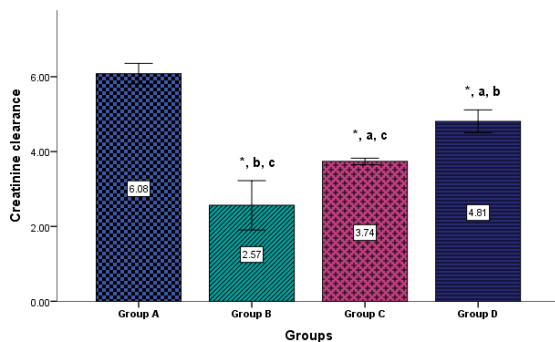


Fig 7: Comparison of Creatinine clearance level in different experimental Groups
 Values are expressed as mean \pm SEM, n = 4.
 * = significantly different from Group A at $p < 0.05$
 a = significantly different from Group B at $p < 0.05$
 b = significantly different from Group C at $p < 0.05$
 c = significantly different from Group D at $p < 0.05$

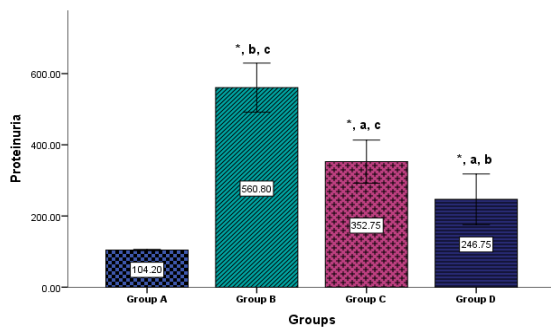


Fig 8: Comparison of Proteinuria level in different experimental Groups
 Values are expressed as mean \pm SEM, n = 4.
 * = significantly different from Group A at $p < 0.05$
 a = significantly different from Group B at $p < 0.05$
 b = significantly different from Group C at $p < 0.05$
 c = significantly different from Group D at $p < 0.05$

4. Discussion

This study has revealed that sustained hyperglycaemia within the tissue of the kidney can cause significant reduction in weight of organ. It has been demonstrated that weight of an organ depends, to a large extent, on the mass of various cells. According to Wolf (2004), hyperglycaemic injury affects all cell types in the kidney including mesangial, vascular endothelia interstitial and fibroblast cells at varying degrees. When compared to the diabetic control group, ten weeks of administration of β -D-glucan polysaccharide to diabetic rat may have caused the increase in kidney weight in a dose-dependent manner. Chang et al. (2011) have reported that *A. polytricha* (unfractionated fungi) is capable of inhibiting lipid peroxidation in body organs thereby restoring their weight.

There are also confirmatory reports by Sherma *et al.* (2001).

The remarkably higher levels of serum SOD, Catalase and melondialdehyde in group B when compared to group A confirm high hyperglycaemic ambience within the renal tissue. It has been documented that β -D-glucan polysaccharide is a strong exogenous source of antioxidant that do not exist as a single form but forms conjugates with other vital bio-molecular elements like amino acid, protein, lipid and nucleic acid residue (Nie et al., 2011). The underlying mechanism could be that β -D-glucan polysaccharide has low molecular weight and more hydroxyl group terminals capable of accepting and eliminating free radicals and these are the most important structural feature that determines the antioxidant capacity of polysaccharides. Liu et al. (2010) reported that peptide moiety of polysaccharide has been found to have high free radical scavenging activity which is a possible reason for low levels of oxidative stress markers in diabetic animals treated with β -D-glucan polysaccharide.

As shown in figure 4, Group A presented a kidney section having prominent renal tubules with an intact glomeruli (figure 4a). Histological section of kidney in diabetic control (figure 4a) group reveals diffused expansion and proliferation of mesangium, thickening of glomerular basement membrane, renal tubular hypertrophy, scanty and sparse interstitial cells with inflammatory infiltrates, distorted Bowman's space and extensive hemorrhage within the mesangial matrix as a result of hyperglycaemic injury which affects all cell types in the kidney. Fioretto and Mauer (2007) reported that glomerular, tubule-interstitial and vascular changes constitute pathogenicity of diabetic nephropathy. In this study, glomerular changes are shown in mesangial cell proliferation and thickening of glomerular basement membrane, changes in the tubulointerstitial compartment included inflammatory infiltrate and tubular hypertrophy while the changes in vascular compartment were notices in the various degrees of hemorrhage within the mesangial matrix. Hyperglycaemia-induced injury results from excessive generation of ROS causing changes in intraglomerular hemodynamics.

Extensive experimental evidence (Aitken and Krausz 2001; Lenzi *et al.*, 1993;) have reported modification of nucleotide bases thereby causing alteration in DNA structure accompanied by deletion, cross linking and chromosomal rearrangement with its attendant consequences that ends in cellular damage.

Findings from this study also show that hyperglycaemia-induced alterations which are signs of kidney damage were minimized and reversed in a dose dependent manner following administration of β -D-Glucan polysaccharide as shown in figures 4c and 4d. Even though a comprehensive investigation into the mechanism of action of β -D-Glucan polysaccharide is still lacking, 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. β -D-Glucan polysaccharide have also been shown to possess lipid peroxidation inhibitory effect which make it capable of preventing damage of cell membrane. Renard et al (2001) reported similar findings.

Renal parameters obtained from the diabetic control group are indicators of renal dysfunction. Results obtained

from renal parameters in different experimental groups show a correlation between these parameters and hyperglycaemia-induced kidney injury. However, these values were reversed following β -D-Glucan polysaccharide administration. Improvement in these renal parameters following administration of β -D-glucan polysaccharide may have reflected the reversal in hyperglycaemia-induced distortion of glomerular, tubulointerstitial and vascular compartments in the kidney tissue.

In conclusion, the findings from this research revealed that β -D-Glucan polysaccharide fractionate of *A. polytricha* was capable of ameliorating hyperglycaemia-induced kidney damage in experimental diabetic nephropathy models. This research therefore suggest that β -D-Glucan polysaccharide may reduce the risk of kidney dysfunction in diabetic men.

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Competing Interest

All authors in this research are not by any means linked to any funding body, and so there was a complete absence of competing interest.

Authors' Contributions

All authors gave equal contribution to this research.

Ethical Clearance

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

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