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Evaluation of the Nephroprotective and Hypoglycemic Effect of Aqueous Extract of Leaf and Root of *Thaumatococcus danielli* on Alloxan-Induced Diabetic Rats

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Abstract:
The management of diabetes without side effects is a major challenge affecting majorities of the world population, thus the constant search for alternative compounds from natural products. This study investigated the nephroprotective and the hypoglycemic effect of aqueous extract of the leaf and root of Thaumatococcus danielli (T. danielli). Rats were assigned to 9 groups (1-9) of four rats each. Each group was made diabetic using Alloxan (150 mg/kg/b.wt) except the control group. The treatment group received 0.5 ml of Metformin (150 mg/kg/b.wt,), 200 and 400 mg/kg/b.wt, of Thaumatococcus danielli aqueous extract of leaf, root, and mixture orally, respectively. After 14 days of treatment, animals were sacrificed, and blood and kidney samples were collected for further analysis.
The lowest reduction in glucose level was 22.65%, which was observed in the diabetic group treated with 200 mg/kg aqueous leaf of T. danielli, while the highest decrease was observed to be 38.63% in the diabetic group treated with 400 mg/kg aqueous root of Thaumatococcus danielli. The diabetic control group had a significantly higher level of sodium and potassium ions. Little changes were observed in chloride, carbonate, and total protein level in the diabetic group compared to the control group; while urea and creatinine levels were higher. Histological alterations such as poor architecture, few collapsed renal tubules, and loss of luminar spaces were observed in control.
From this study, it can be inferred that aqueous extracts of the leaves and roots of Thaumatococcus danielli and their combination have antihyperglycemic activity as well as modulating renal indices and diabetes-induced nephropathy.

Keywords: *Thaumatococcus danielli, nephroprotective, metformin, electrolytes*

1. Introduction

Diabetes is associated with a substantially increased risk of mortality, particularly due to cardiovascular disease (Seshasai *et al.*, 2011). Beyond medications that control glycemia and blood pressure, only medications that inhibit the Renin–angiotensin System (RAS) have had robust renoprotective effects in randomized, controlled trials. A recently published secondary analysis showed the nephro-protective effects of empagliflozin, an inhibitor of sodium–glucose cotransporter 2 (SGLT2) in type 2 diabetes (Wanner *et al.*, 2016). Long-term complications of diabetes mellitus include:

- Retinopathy,
- Nephropathy,
- Neuropathy, and
- An increased risk of cardiovascular disease

The persistent state of hyperglycaemia seen in diabetes predisposes the person to diabetes-associated organ dysfunctions, especially in the eyes, kidneys, nerves, heart, and blood vessels (Das & Barman, 2012).

Diabetic nephropathy (DN) is one of the major micro vascular complications of diabetes mellitus, with its development being confirmed through significant enhancement in the level of blood urea nitrogen (BUN), glycated serum protein (GSP), and serum creatinine (Scr) (Ahmed *et al.*, 2014).

The necessity of developing an effective treatment to control this metabolic disorder is of paramount importance. To achieve the objective, studies on experimental albino rats were performed by inducing diabetes with hyperglycemic drug: Alloxan in different doses per the rat's body weight to achieve a stable diabetic state (Roja *et al*., 2017).

Presently available therapy for diabetes mellitus includes insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazones, and GLP-I agonists are not without unwanted side effects (Priscilla, 2013). Some of these effects may increase plasma levels of homocysteine, which is a very significant risk factor for cardiovascular disease. This has encouraged the use of medicinal herbs for the treatment of diabetes, especially by local traditional medicine practitioners in many developing countries (Das & Barman, 2012).

Thaumatococcus danielli, a plant species from West Africa, is a medicinal plant of great importance. It is commonly used in folk medicines to cure or manage a wide range of diseases, including insanity and pulmonary problems, in addition to being used as a laxative, sedative, and for the treatment of diabetes in the Western part of Nigeria (Lim, 2012). Ethno-pharmacological studies demonstrate the use of these plants in the management of diabetes (Olorunnisola *et al*., 2016). The anti-diabetic study on *T. danielli* in alloxan-induced diabetic rats conducted by Emudainohwo (Emudainohwo *et al.,* 2015) revealed the significant potential of the plant as a new source of the hypoglycemic drug while failing to address the possible protective role of the plant on renal functions often impaired in diabetes. With this backdrop, the nephro-protective effect of leaf, root, and combined extract of Thaumatococcus danielli in alloxan-induced diabetes rats was investigated.

2. Materials and Methods

2.1. Plant Collection

Fresh leaves of the *Thaumatococcus danielli* leaves and roots were obtained from NIHORT, Ibadan.

2.2. Preparation of Plant Extract

Fresh leaves and roots of *Thaumatococcus danielli* were crisply air-dried and powdered. 400 g of powdered leaf and 100g of powdered root were macerated in 2L and 1L of distilled water, respectively, for 72 hours. The mixtures were filtered, and their filtrate was concentrated using a rotary evaporator and stored appropriately (Folurunsho *et al*., 2019).

2.3. Animal Experiment

36 male rats were obtained and grouped as follows:

- Grp1 (Normal Contol),
- Grp2 (Diabetic control),
- Grp 3 (Diabetic rats treated with aqueous leaf at 200mg/kg),
- Grp 4 (Diabetic rat treated with aqueous leaf at 400mg/kg),
- Grp 5 (Diabetic rats treated with an aqueous root at 200mg/kg),
- Grp 6 (Diabetic rat treated with an aqueous root at 400mg/kg),
- Grp 7 (Diabetic rats treated with an aqueous mixture of leaf and root at 200mg/kg),
- Grp 8 (Diabetic rats treated with an aqueous mixture of leaf and root at 400mg),
- Grp 9 (Diabetic rats treated with metformin)

2.4. Induction of Diabetes Milletus

The animals were deprived of food but free access to water 24hrs prior to induction of diabetes. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate at a dose of 150mg/kg prepared in stock of 1000mg/33ml (30mg/ml). 2 days after induction of diabetes animal with fasting blood glucose above 200mg/dl were considered as diabetic and were used in the study (Stanley & Venugopal, 2001).

2.5. Collection of Sample

On the 15th day, the animals were sacrificed, and their blood was obtained by cardiac puncture technique using diethyl ether as an anesthetic agent. The blood samples were stored in non-heparinized tubes (Wallenfel *et al*., 2005). They were allowed to clot for 1h, 30 mins, and afterward centrifuged at 5000rpm for five minutes to remove cells and recover serum for the biochemical assays. The kidneys were surgically removed and de-capsulated, then cleansed of blood with 0.25 M sucrose solution and afterward fixed in 10% formaldehyde.

2.6. Biochemical Assays

Electrolytes (Na+, K+, Cl, and HCO_3^-), creatinine, urea, and total proteins were estimated according to the manufacturer's instructions on the assay kits (Olubukola *et al*., 2017).

2.7. Histopathological Study

The fixed kidney tissues were sectioned (5-micron thickness), and sections stained with Hematoxylin and Eosin (H&E) according to Conn (Conn, 2006) procedure, and photomicrographs were developed (x 400).

2.8. Data Analysis

Data generated were presented as mean ± standard deviation of replicate measurements; analysis of variance was used to assess significant differences in the means of the measured parameters in the group treatments, while Turkey's post hoc test was used to identify specific differences between groups. The probability level at $p<0.05$ was considered significant. Statistical analysis was performed using SPSS version 20.

3. Results

3.1. Effect of Alloxan Administration and Aqueous Extract of Thaumatococcus danielli on the Body Weight in (g) Experimental Rats

The weights of Alloxan-induced diabetic rats 2 days after induction reduced significantly, as shown in table 1, while there was a significant ($p<0.05$) time-dependent modulation by all extract treatments except the standard drug and 400 mg/kg bwt of the combined aqueous root and leaf extract of *T. danielli.*

Treatment	Weight b4 Induction	Weight 2 days after	Weight after Treatment
Control Group	146 ± 3^{a}	150 ± 3^{a}	167 ± 3^{b}
Alloxan Untreated	174 ± 1^{b}	170 ± 2^{b}	163 ± 4^{a}
Alloxan+200 mg/kg TDLE	132 ± 2^{b}	127 ± 2^{a}	133 ± 2^{b}
Alloxan+400 mg/kg TDLE	160 ± 3^{b}	154 ± 3^{a}	166 ± 3^{c}
Alloxan+200 mg/kg TDRE	176 ± 4^{b}	170 ± 1^{a}	179 ± 1^{b}
Alloxan+400 mg/kg TDRE	133 ± 4^{ab}	128 ± 3^{a}	139 ± 2^{b}
Alloxan+200 mg/kg TDL/RE	144 ± 3^{ab}	136 ± 4^{a}	145 ± 4^{c}
Alloxan+400 mg /kg TDL/RE	156 ± 7^{a}	151 ± 8^{a}	161 ± 7^{a}
150 mg Metformin	191 ± 5^{a}	188 ± 5^{a}	196 ± 4^{a}

Table 1: Weight Changes Prior to and Following Alloxan Induction and Extract Treatment

Mean with different alphabets in the same row are significantly different ($p<0.05$); n = 4

Key: TDLE – Thaumatococcus danielli Leaf Extract; TDRE - Thaumatococcus danielli Root Extract; TDL/RE – Thaumatococcus danielli Leaf and Root Extract combination

3.2. Effect of Alloxan Administration and Aqueous Extract of Thaumatococcus Danielli on the Fasting Blood Sugar of the Experimental Rats

The fasting blood sugar (FBS) levels of the rats and the estimated increase/decrease in blood glucose levels after treatment have been determined (Table 2). The FBS after alloxan induction was 76-413mg/dl, while the FBS after 14 days of treatment was 75-351mg/dl. The diabetic group treated with 200 mg/kg body weight aqueous leaf extract of Thaumatococcus danielli had the lowest glucose reduction of 22.65%, while the diabetic group treated with 400 mg/kg bwt aqueous root extract of Thaumatococcus danielli had the highest decrease of 38.63%.

Treatment	FBS after Induction	FBS after Treatment	% Change
Control Group	76 ± 9^{a}	75 ± 11^{a}	-1.32
Alloxan Untreated	274 ± 81^{b}	351 ± 74^{c}	+28.10
Alloxan+200 mg/kg TDLE	309 ± 12^{bc}	239 ± 10^{b}	-22.65
Alloxan+400 mg/kg TDLE	391 ± 19^{c}	264 ± 21^{bc}	-32.48
Alloxan+200 mg/kg TDRE	396 ± 27^{c}	301 ± 23^{bc}	-23.98
Alloxan+400 mg/kg TDRE	409 ± 16^{c}	251 ± 12^{bc}	-38.63
Alloxan+200 mg/kg TDL/RE	345 ± 50^{bc}	267 ± 51^{bc}	-22.70
Alloxan+400 mg /kg TDL/RE	333 ± 47^{bc}	231 ± 33^{b}	-30.63
150 mg Metformin	413 ± 83^{c}	266 ± 75^{bc}	-35.59

Table 2: The Effect of Alloxan Administration and Thaumatococcus danielli Aqueous Extract on the Experimental Rats' Fasting Blood Sugar Level (mg/dl)

3.3. The Effect of Thaumatococcus danielli Aqueous Extracts on Renal Indices in Alloxan-Induced Diabetic Rats

Table 3 shows the effect of graded doses of Thaumatococcus danielli aqueous extracts on renal indices. The sodium ion ranged from 137 to 145mmd/l, the potassium ion from 3.6 to 4.5mmd/l, the chloride ion from 101 to 111mmd/l, and the hydrogen carbonate ion from 21 to 24mmd/l. The urea concentration ranged from 24-69 mg/dl, the creatinine concentration ranged from 0.6-1.3 mg/dl, and the total protein concentration ranged from 6.2-7.2 mg/dl. The analysis of variance results indicates significant differences between the treated, diabetic, and control groups.

Treatment	Na^+	K^+	Cl^-	HCO_3^-*	Urea	Creatinine	Total Protein
Control Group	139 ± 1^{ab}	3.9 ± 0.1^{abc}	105 ± 0^{abc}	23 ± 1	27 ± 1^{a}	0.6 ± 0.0^{a}	6.9 ± 0.1^{bc}
Alloxan Untreated	145 ± 1^{b}	4.5 ± 0.1^{d}	110 ± 0^{bc}	21 ± 1	69 ± 8^{c}	1.3 ± 0.1^{c}	6.2 ± 0.1^{a}
Alloxan+200 mg/kg TDLE	143 ± 0^{ab}	4.3 ± 0.1^{cd}	110 ± 0^{bc}	22 ± 1	50 ± 3^{b}	1.0 ± 0.1^{b}	6.6 ± 0.1^{ab}
Alloxan+400 mg/kg TDLE	137 ± 1^{a}	4.2 ± 0.1^{bcd}	101 ± 1^{a}	21 ± 1	27 ± 1^{a}	0.7 ± 0.1^{a}	6.9 ± 0.1^{bc}
Alloxan+200 mg/kg TDRE	140 ± 1^{ab}	4.1 ± 0.1^{abcd}	103 ± 4^{ab}	23 ± 2	29 ± 3^{a}	0.7 ± 0.1^{a}	7.1 ± 0.1^{bc}
Alloxan+400 mg/kg TDRE	139 ± 4^{ab}	3.6 ± 0.2^{a}	103 ± 4^{ab}	24 ± 3	24 ± 5^{a}	0.6 ± 0.1^{a}	6.6 ± 0.3^{ab}
Alloxan+200 mg/kg TDL/RE	143 ± 1^{ab}	4.2 ± 0.1^{bcd}	111 ± 1^{c}	22 ± 1	39 ± 1^{ab}	0.7 ± 0.1^{a}	7.2 ± 0.0^{c}
Alloxan+400 mg /kg TDL/RE	139 ± 2^{ab}	4.1 ± 0.2^{abcd}	105 ± 0^{abc}	24 ± 1	27 ± 3^{a}	0.7 ± 0.1^{a}	6.9 ± 0.1^{bc}
150 mg Metformin	140 ± 2^{ab}	3.7 ± 0.1^{ab}	103 ± 4^{ab}	23 ± 1	26 ± 4^{a}	0.6 ± 0.0^{a}	6.8 ± 0.1^{bc}

*Table 3: The Effect of Thaumatococcus danielli Aqueous Extracts on Renal Indices in Alloxan-Induced Diabetic Rats Means with different alphabets in the same column are significantly different ($p<0.05$); n = 2; * not significant ($p>0.05$*

3.4. Effect of Thaumatococcus danielli Aqueous Extracts on Histology of the Kidney

The normal control group did not exhibit any abnormal changes in the renal histopathology of the rats, as shown in figures 1 and 2. The rats showed normal glomerular size, basement membrane thickness, and architecture; the groups were induced with alloxan. On the other hand, it showed abnormal changes characterized by poor architecture, a few collapsed renal tubules, and loss of luminar spaces. Depending on the extract dose, the architecture of the treatment groups and the standard drug-treated group ranged from poor to moderately normal.

In comparison to the control group, the histopathological study of Alloxan-induced diabetic rats revealed vascular wall thickening, necrosis or degeneration, and loss of luminar interstitial, inflammation, and moderate degrees of glomerular dilation

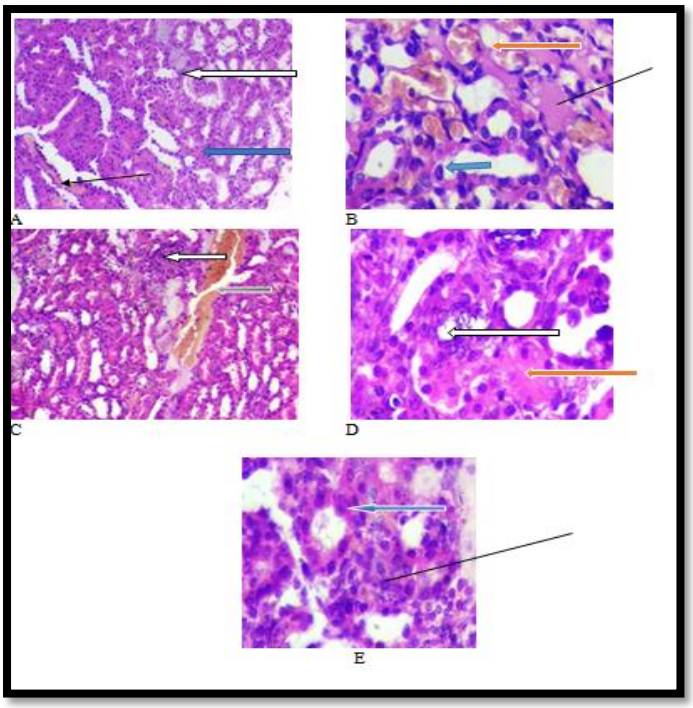

Figure 1: Photomicrograph of (A) Normal control; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), the interstitial spaces appear normal (slender arrow), (B) Diabetic Group; few renal tubules appear collapsed with loss of luminar spaces normal (blue arrow), the interstitial spaces show mild congestion (red arrow) and mid fluid accumulation in the interstitium of medulla (slender arrow), (C) Diabetic/200 mg/kg TDLE; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow and moderately congested vessel (green arrow), (D) Diabetic/400 mg/kg TDLE; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), few renal tubules appear collapsed and mildly degenerated (red arrow), (E) Diabetic/200 mg/kg TDRE; the renal tubules appear normal (blue arrow), the interstitial spaces show severe infiltration of inflammatory cells (slender arrow).

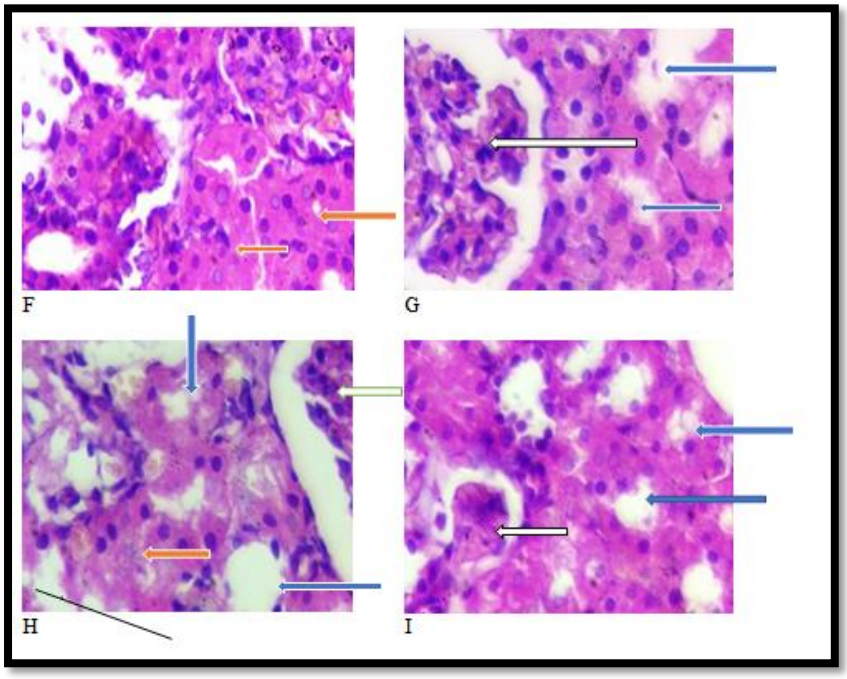

Figure 2: Photomicrograph of (F); some of the renal tubules appear normal (blue arrow), and others are collapsed with the loss of luminar spaces (red arrow), Diabetic/400 mg/kg TDRE (G) Diabetic/200 mg/kg TDL/RE; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow) (H) Diabetic/400 mg /kg TDL/RE; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), few tubules are attenuated (red arrow), the interstitial spaces appear normal (slender arrow), (I) Diabetic/150 mg Metformin; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow).

4. Conclusion

From the result of this work, it can be deduced that aqueous *Thaumatococcus danielli* leaves, roots, and their combination have antihyperglycemic activity as well as modulating renal indices and diabetes-induced nephropathy.

200 mg/kg bwt aqueous leaf extract and 400 mg/kg bwt combined extract are sufficient to significantly reduce blood sugar levels.

400mg/kg bwt root extract is also promising, as it is capable of reducing blood sugar over a wide range. It is also suggested that the plant can aid in the loss of weight associated with diabetes while also improving renal histopathology indices. The study supports the use of T. daniellii in folk medicine in the treatment and management of diabetes and its complications.

5. Recommendation

It is recommended that more research be conducted to isolate and characterize hypoglycemic and nephroprotective compounds from *T. danielli* aqueous leaf and root extracts. It should also be noted that the treatment with this plant should last longer than 14 days to have the maximum effect on this plant.

It is also recommended that this plant extract's dosage standardization be established for it to be easily adopted in complementary medicine.

6. References

i. Ahmed, D. Kumar, V. Verma, A. Gupta, P.S. Kumar, H. Dhingra, V. Mishra, V. & Sharma, M. (2014) Anti-diabetic, renal/hepatic/pancreas/cardiac protective and anti-oxidant potential of methanol/dichloromethane extract of Albizzia Lebbeck, Benth. Stem bark (ALEx) on streptozotocin induced diabetic rats. *BMC Complementary and Alternative Medicine*.; 14(243): 4–17.

ii. Emudainohwo, J.O.T. Erhirhie, E.O. Moke, E.G. & Ejebe, D.E. (2015) Hypoglycemic Effect of ethanol leaf extract of Thaumatococcus danielli in Alloxan Induced Diabetic Wistar Rats. *10SR. J. Pharm. Biol. Sci*. 10(2): 59–64.

iii. Lim, T.K. (2012) *Edible Medicinal and Non-Medicinal Plants*: Volume 3, Fruits. Springer Science Business Media B.V.

iv. Lorke, D. (2005): A New Approach to Tropical Acute Toxicity Testing. *Arch Toxicol*; 53: 275–287.

v. Olorunnisola, O.S. Adetutu, A. Owoade, A.O. Okoh, O.O. Oyewo, E.B. & Adegbola, P. (2016) Ethno pharmacological and in-vitro anti-diabetic study of some medicinal plants commonly used in Ogbomoso, South Western Nigeria. *J. Appl. Biosci*. 105.

vi. Stanley, P.M.P. & Venugopal, M.P. (2001): Anti-oxidant action of Tinospora cordifolia root extract in alloxan diabetic rats. *Phytother Res*, 15: 213–218.

vii. Seshasai, S.R. Kaptoge, S. Thompson, A. Di Angelantonio, E. Gao, P. Sarwar, N. Whincup, P.H. Mukamai, K.J. & Danesh, J. (2011) emerging risk Factors Collaboration; Diabetes mellitus, Fasting glucose and risk of causing specific death. *N Engl J Med* 364; 829–841.

viii. Wallenfels, B. Eisele, & Bender, H. (2005), A spectrophotometric method for measuring the activity of amylase. Carbohydrate Res., 61, 359–364.

ix. Wanner, C. Inzucchi, S.E. Lachin, J.M. Fitchett, D. Eynatten, M. Mattheus, M. Johansen, O.E. Woerle, H.J. Broedl, U.C. & Zinman, B. (2016) Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med*; 375:323–34

x. Das, & Barman, S. (2012). Anti-diabetic and antihyperlipidemic effects of ethanolic extract of leaves of *Punica granatum* in Alloxan-induced Non-insulin-dependent diabetes mellitus albino rats. *Indian Journal of Pharmacol*ogy 44: 219–224.

xi. Roja, R.C. Akella, S.R. & Vijayaraghavan, R. (2017) A histological study of alloxan-induced diabetes on experimental male wistar rats, *National journal of pharmacy and pharmacology* Vol 7 Issue 12.

xii. Priscilla, H. Elemo, B.O. Fatai, A.A. Bankole, H.A. & Kazeem, M.I.(2013), The Role of Anti-obesity Drugs in Patients with Type 2 Diabetes.*US Endocrinology*. 9(2), 101–7.