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In vitro anti-diabetic effect and cytotoxicity of South African *Ipomoea oblongata*

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ABSTRACT

Ipomoea oblongata is a popular plant known for its diverse therapeutic uses in South African folk medicine but currently has limited empirical evidence to support some of these uses. In the current study, we evaluated the *in vitro* anti-diabetic and cytotoxic effects of the root extracts of *Ipomoea oblongata*. Methanol and water extracts were tested for anti-diabetic activity using glucose uptake/utilisation response in muscle (C2C12) and Chang liver cell lines. Water extract demonstrated a noteworthy (182%) anti-diabetic activity in muscle cell lines which was similar to the effect exerted by insulin (192%, positive control). No noteworthy glucose uptake/utilisation occurred with the Chang liver cell lines. Generally, *Ipomoea oblongata* extracts were relatively safe based on the response in the MTT assay against muscle (C2C12) and Chang liver cell lines. The noteworthy *in vitro* anti-diabetic effect and absence of cytotoxic effect against both cell lines provide preliminary empirical evidence that supports the utilisation of *Ipomoea oblongata* as a popular herbal medicine in folk medicine.

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Introduction

Ipomoea oblongata E. Mey. ex Choisy (synonym: *Turbina oblongata*) is a member of the family Convolvulaceae. In this family, the genus *Ipomoea* is one of the largest with approximately 600 species existing throughout tropical and subtropical regions of the world (Srivastava and Rauniyar 2020), where many of these species are often utilised as ornamentals, food, religious and therapeutic purposes (Srivastava 2017). In South Africa, *Ipomoea oblongata* is found in the majority of the provinces especially the Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga and North West (Foden and Potter 2005). Different parts of *Ipomoea oblongata* are known for diverse medicinal conditions (e.g. wound-healing, arthritis, reproductive ailments, respiratory infections, lymphatic filariasis, inflammation, asthma, kidney failure and hypertension) among various ethnic groups in southern African countries especially South Africa (Sobiecki 2002; Tshikalange et al. 2016; Komoreng et al. 2017; Polori et al. 2018) and Lesotho (Seleteng Kose et al. 2015; Moteetee and Seleteng Kose 2016). Some *Ipomoea* species have been investigated for biological efficacies, in an attempt to explore their potential for mitigating different disease conditions (Srivastava and Rauniyar 2020). However, only limited studies have been recorded in terms of the biological activities of *Ipomoea oblongata*. Recently, Lall et al. (2019) demonstrated the antioxidant, anti-tyrosinase and anti-acne (*Cutibacterium acnes*) potential of the root-bark ethanol extract. In addition, it had moderate cytotoxic effect against human keratinocyte cells under *in vitro* conditions. In our previous study (Polori et al. 2018), we reported a noteworthy free-radical scavenging activity (antioxidant effect) of the root extract (methanol) of *Ipomoea oblongata* and revealed the presence of diverse phytochemicals. Given the multi-purpose uses and popularity of *Ipomoea oblongata* among traditional healers in the Free State Province of South Africa (Polori et al. 2018), the current study investigated the *in vitro* anti-diabetic and cytotoxicity of *Ipomoea oblongata* root extracts.

Materials and methods

Source and preparation of plant material

Ipomoea oblongata was generously donated by one of the traditional medical practitioners of Kopanang Dingaka association in Thaba-Nchu. After collection, a voucher specimen (KT001/2013) was deposited at the Central University of Technology Free State following authentication by an expert at the National Botanical Garden in Bloemfontein, South Africa. Plant material (roots) were oven-dried at

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40 °C for 5 days, crushed into a fine powder and stored in a plastic jar. For the preparation of water and methanol extracts, 10 g of *Ipomoea oblongata* roots were weighed and soaked for 72 h in 150 mL of the different solvents and stirred using a rotary shaker. After filtering through (Whatman No. 1) filter paper, methanol solvent extract was dried at 40 °C after removing the excess solvent using a rotary evaporator while the water extract was freeze-dried.

In vitro *anti-diabetic assay*

In vitro anti-diabetic test was based on glucose uptake model in C2C12 (muscle) and Chang (liver) cells as described by van de Venter et al. (2008). Briefly, the cells (C2C12) were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum at 37 °C in humidified air with 5% CO_2. The muscle (C2C12) cells were seeded at a density of 4000 cells per well into a 96-well culture plate and cultured for 3 days. After differentiation, cells were acutely exposed for 3 h to methanol and water and decoction extracts at concentrations of 50 μg/mL, metformin (1 μM) and insulin (1 μM). The solvent control (DMSO/water) was added to the modified DMEM supplemented with 8 mM of glucose. The glucose concentrations remaining in the wells were determined using a commercial fluorimetric kit. The measurement of glucose concentration in the media was determined using a glucose oxidase fluorimetric assay. Fluorescence was measured at Ex/Em = 535/587 nm using a BioTek FX800 fluorimeter (BioTek Instrumentation Inc., Vermont, USA).

In vitro *cytotoxicity assay*

In order to determine the safety of the plant extract, we used MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium-bromide assay on the viability of two cell lines namely: C2C12 muscle and Chang liver cell lines. The MTT assay is a colorimetric method that measures the reduction of the yellow MTT colour by mitochondrial dehydrogenase to dark purple formazan crystals in living cells (Mosmann 1983; Ulukaya et al. 2008). The solubilized formazan precipitate was measured using a spectrophotometer at 570 nm. The cell passage number was below 10 and the samples were tested in triplicate.

Data analysis

We subjected the data (from the anti-diabetic and cytotoxicity tests) to one-way analysis of variance (ANOVA) and separated the mean at $p \leq 0.05$ using the Bonferroni's Multiple Comparison Test. Furthermore, as recommended by van de Venter et al. (2008), a scoring system was used to evaluate the overall potential of each solvent extract to increase glucose utilisation in different cell types without adverse toxic effects.

Results

In vitro *anti-diabetic response*

Based on the glucose uptake response (%), water extract showed noteworthy *in vitro* anti-diabetic activity in C2C12 muscle cell lines which was similar to that of insulin and metformin (**Table 1**). On the other hand, glucose uptake in the Chang liver cancer cell lines was generally low. Response in water (88%) and methanol (99%) extracts were similar to the vehicle control. Relative to the water and methanol extracts, metformin and insulin had a significantly higher levels of glucose uptake of Chang cells.

In vitro *cytotoxicity response*

Both water and methanolic extract of *Ipomoea oblongata* had cell viability below 100% (reduced MTT activity by >15%), thus suggestive of low level of cytotoxicity **(Supplementary Figure S1)**. No significant difference was observed in terms of the cell variability when both cell lines were cultivated using test extracts, positive control and solvent.

Table 1
Effect of *Ipomoea oblongata* on glucose uptake/utilisation (%) using two cell lines as a measure of *in vitro* anti-diabetic activity. Value are reported mean (standard error) and n =3. In each column, mean value with different letter(s) are significant ($p<0.05$) different based on Bonferroni's Multiple Comparison Test. DMSO = Dimethyl sulfoxide.

Sample	C2C12 muscle cell line	Chang liver cell line
Ipomoea oblongata methanol extract	66±1.65 d	99±1.70 c
Ipomoea oblongata water extract	182±27.16 a	88±9.84 c
Insulin	192±19.3 a	114±1.35 ab
Metformin	157±19.7 ab	116±0.57 a
20% DMSO	100±0.94 cd	100±4.20 c

Based on the applied scoring systems, water extract was preferred to the methanol extract relative to the two cell lines used in the current study **(Supplementary Table S1)**. This suggests that the water extract demonstrated *in vitro* anti-diabetic effects without causing any significant inhibition of the growth of the tested cell lines.

Discussion

Globally, the increasing incidence of diabetes mellitus remain evident and often considered as one of the most prevalent and costly chronic disease (Leon and Maddox 2015). As a result, research interest on medicinal plants and their exploration for their anti-diabetic properties have continuously been pursued (Patel et al. 2012a; Nyakudya et al. 2020). Based on increasing evidence (Nyakudya et al. 2020), South African rich biodiversity holds a great potential for the development of novel plant-derived medicines for the management of metabolic syndrome including diabetes and associated complications. Some South African plants evaluated for anti-diabetic activity have demonstrated promising effects thereby suggesting their important role as alternative for mitigating the increasing burden on the health care systems especially in rural areas (van de Venter et al. 2008; Nyakudya et al. 2020). As an indication of the increasing inventory of anti-diabetic plants in South Africa, we demonstrated the *in vitro* anti-diabetic effect of the water extract of *Ipomoea oblongata* roots. As highlighted by Srivastava and Rauniyar (2020), a number of *Ipomoea* species have demonstrated anti-diabetic effects based on different test models and mechanisms. The regulation of plasma glucose levels and achieving insulin derivative effects remain a common mechanism for treating diabetics (López-Viseras et al. 2014; Kerru et al. 2018). Particularly, this is often linked to type II diabetes which is characterised by hepatic and peripheral (muscle and adipose tissue) insulin resistance (Singh et al. 2013). In the current study, glucose uptake/utilisation by the water extract in the C2C12 muscle cells was increased by over 182%, which is comparable to both insulin and metformin (positive control). On the other hand, Chang liver cell line was not a suitable model based on the low glucose uptake/utilisation in both water and methanol extracts. Chang cells, in contrast to C2C12 cells, do not express the insulin-responsive glucose transporter glucose transporter 4 (GLUT4), which is expressed primarily in muscle and adipose tissue and recognised as the major insulin-responsive glucose transporter isoform (Watson and Pessin 2001). This explains the low response of Chang cells as they are less sensitive to acute insulin stimulated glucose uptake. Glucose uptake and insulin release are suggestive of hypoglycemic effects, which may be due to the action on hepatic gluconeogenesis or glycogenesis (Patel et al. 2012a). Based on the noteworthy glucose uptake by water extract of *Ipomoea batata* roots using C2C12 cell line, the potential mechanism of action may be related to the reduction of insulin resistance and glucose level in the test system (Patel et al. 2012b).

Given that antioxidant defences and cellular redox status are considered as central player in diabetes and its complications (West 2000), plants that exert potent antioxidant activity which are often attributed to the presence of phenolic compounds may be relevant as anti-diabetic agent. Evidence of the antioxidant effect of *Ipomoea oblongata* has been demonstrated in previous studies (Polori et al. 2018; Lall et al. 2019). Generally, the antioxidant effects are attributed to the protective role of phytochemicals which are often present in many medicinal plants (Zhang et al. 2015). It has been established that bioactive compounds such as polyphenols, alkaloids, flavonoids, glycosides, carotenoids, terpenoids and coumarins greatly contribute to the anti-diabetic activity of medicinal plants, causing reduction in blood glucose levels (Patel et al. 2012a). As a result, there is increasing effort geared at screening for chemical entity from medicine plants as a promising approach for new drug discovery to treat the diabetic and associated complications (Singh et al. 2013). On the basis of findings by Polori et al. (2018), the presence of valuable phytochemicals in *Ipomoea oblongata* possibly contributed to the ability of this plant to exert anti-diabetic activity. It has been postulated that the anti-diabetic effect might be attributed to amelioration of persistent hyperglycemia, oxidative stress and modulation of various metabolic pathways involved in the pathogenesis of diabetic complications (Singh et al. 2013).

Generally, the cytotoxic and toxicological effects of plant extracts have been overlooked because of the belief that medicinal plants have better compatibility with the human body owing to their perceived fewer side effects. However, the safety evaluation remain an important requisite in order to facilitate the integration of traditional medicine into public health programmes (McGaw et al. 2014; Moyo et al. 2015). As recently demonstrated by Lall et al. (2019), ethanol extract of *Ipomoea oblongata* root-bark had moderate cytotoxic effect (IC_{50} = 52.70 ± 8.60 μg/mL) against human keratinocyte (HaCat) cells when measured using 2,3-Bis-(2-methoxy-4 nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt (XTT) cell viability reagent. In the current study, we observed that the both water and water extracts had no significant adverse effect on the viability of C2C12 muscle and Chang liver cells at the tested concentrations. Even though the cytotoxicity was low in the current study, it is essential to explore other test systems given the inherent limitations associated with the use of many *in vitro*-based test model (Twilley et al. 2020). Based on the scoring system proposed by van de Venter et al. (2008), water extract had higher total score than the methanol extract. This suggests that the water extract is relative efficient and safe based on the two cell lines used in the current study. Even though the total score was generally low, this may be attributed to the weak glucose response in the Chang cell line.

Conclusions

In the current study, we demonstrated the noteworthy *in vitro* anti-diabetic activity *Ipomoea oblongata* water extract based on the glucose uptake/utilisation potential. In addition, the relative safety of the extracts in form of the high viability of C2C12 muscle and Chang liver cell lines in the MTT assay is encouraging. Overall, the current findings contribute to the empirical data on the increasing therapeutic effects of *Ipomoea oblongata*, which remain widely used among the traditional healers in Free State Province of South Africa. On the basis of the promising anti-diabetic effect, the isolation and identification of the active compound(s) as well as *in vivo* model for the evaluation of the anti-diabetic activity is recommended in future research.

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Author Contributions

Conceptualization, K.L.P. and S.S.M.; methodology, K.L.P.; formal analysis, K.L.P., S.S.M. A.O.A.; investigation, K.L.P.; resources, S.S.M.; writing—original draft preparation, K.L.P., A.O.A.; writing—review and editing, K.L.P., S.S.M., A.O.A; supervision, S.S.M.; project administration, S.S.M.; funding acquisition, S.S.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

We hereby declare no conflict of interest with regards to the current research. CUT, MRC and NWU had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2021.06.024.

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