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RESEARCH ARTICLE

In vivo antidiabetic potential of standardized *Gymnocarpos decandrus* Forssk. Extract

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Abstract

Background *Gymnocarpos decandrus* (Caryophyllaceae) is a well-known wild plant used as a food for grazing animals. Recently it showed potent antidiabetic potential beside its established anti-inflammatory, analgesic and diuretic activities. *G. decandrus* antidiabetic potential was reported through *in-vitro* models and resulted in promising α-amylase, α-glucosidase and antiviral *Coxsackie B4* inhibitory activities; however no *in-vivo* studies were conducted.

Purpose This study aims to examine *Gymnocarpos decandrus* ethanol extract (GDEE) safety and to evaluate its in vivo antidiabetic potential.

Method Adult albino rats were injected intraperitoneally with alloxan to induce diabetes mellitus and the glucose level was measured after two and four weeks against metformin as a standard drug. Additionally, GDEE characterization and standardization were carried out.

Results GDEE LD_{50} was up to 5.8 mg/kg and exhibited significant antidiabetic activity 77.17% comparable to the standard drug metformin. Its total phenolics, and flavonoids amounted 127.2 ± 0.23 and 85.5 ± 0.21 mg/g respectively. Vitexin was used as a marker compound for GDEE (140.70 mg/100 gm).

Conclusion This study represents the sole in vivo scientific validation of *G. decandrus* recently documented in vitro antidiabetic potential.

Keywords *Gymnocarpos decandrus* · In vivo · Antidiabetic · Alloxan · Vitexin

Introduction

The International Diabetes Federation reported that diabetes mellitus (DM) affects more than 366 million worldwide and is expected to increase by the year 2030 to 552 million. Diabetes affects more than fourteen million people in Africa. West Africa was documented to exhibit the highest prevalence of DM, with Nigeria and Côte d'Ivoire ranking first and second, respectively. In South Africa tops and Southern Africa, the list followed by the Democratic Republic of Congo. Cameroon recorded the highest cases numbers in the Africa central countries [1]. Egypt is expected to have at least 8.6 million case of DM by the year 2030 [2]. The prevalence of diabetes in Egypt is rising. It is one of the most common cause of disability and premature mortality in such developing country [3].

The World Health Organization (WHO) reported that eighty percent of Asia and Africa population use traditional medicine for primary health care. Similarly developed countries represent 70–80% of the populations that use alternative or complementary

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medicine. Recently, research has focused on medicinal plants which was proved to provide reliable, effective, and inexpensive treatment [4]. The use of medicinal plants as antidiabetic candidates gained wide popularity in the last decades [5–7].

Exploring medicinal plants and their metabolites for diabetes management is not just a way to discover safer pharmaceuticals alternatives, but also is an attempt to find a natural effective affordable drug especially in developing countries.

Gymnocarpos decandrus Forssk. (Caryophyllaceae) is distributed in Middle East and North Africa. *G. decandrus* is a shrublet with erect stem, up to 45 cm tall [8]. It is widely distributed in Egypt north coast where it is used as a food for grazing animals [9]. New reports focused on *G. decandrus* aerial parts chemical profile [10, 11]. Recently the plant was proved to exhibit potent anti-inflammatory, analgesic, diuretic, α-amylase, α-glucosidase inhibitory, antitumor, antioxidant, antimicrobial, anticoagulant, and anti *Coxsackie B4* virus activities [10, 12, 13]. The plant previously reported secondary metabolities [10, 14–17]; apigenin, luteolin, epiafzelechin, catechins, epicatechins, daphnoretin, protocatechuic acid, vitexin and quercetin exhibited in vitro antidiabetic potential via inhibition of α-amylase [11], α-glucosidase and *Coxsackie B4* virus which destroy insulin-producing pancreatic beta cells [10]. Its *CoxB4* and digestive enzymes (α-amylase and α-glucosidase) inhibitory activities indicates its ability to manage both types of DM [18–20].

All these reports did not report the plant's safety/toxicity which is essential preliminary step for drug discovery and development. The formerly mentioned studies just reported the in vitro activities which are target-based approaches. The target-based approaches have been dominating early drug discovery in the past decades, which is concurrent with the pharmaceutical research and development productivity crisis [21–23]. This single target-base screen may de facto be inactive as it chooses target (protein /enzyme) which may induce undesired toxicity and may be unessential to disease pathogenesis [24, 25]. In addition, the drug usually displays clinically relevant polypharmacology due to its specific binding to more than one target [26, 27]. In turn, examining the in vivo potential of a newly explored drug is a must preliminary step for drug development. This study targets evaluation of *G. decandrus* in vivo antidiabetic activity and standardization of its crude extract in order to provide more specific and detailed scientific evidence for its use in management of DM.

Material and methods

Plant material and *G. decandrus* ethanol extract (GDEE) preparation

G. decandrus flowering aerial parts were collected from the western Mediterranean coastal region (Mersa Matrooh-Alexandria road) during April, 2018. It was identified by Prof. Dr. Azza El Hadidy, Professor of Taxonomy and Flora- Herbarium, Faculty of Science, Cairo University. Voucher specimen (code number 7.12.15.1) was deposited at Pharmacognosy Department Herbarium, Faculty of Pharmacy, Cairo University. The *G. decandrus* ethanol extract (GDEE) previously prepared [10] was used.

Estimation of total phenolics and flavonoids

Total phenolic content (TPC) estimation

TPC in GDEE was estimated with Folin-Ciocalteu reagent using the method of [28]. Gallic acid was used as a standard. Sample spectral absorbance was measured at 765 nm using UV/visible light and the TPC was calculated from the standard calibration curve and was expressed as mg/g of gallic acid equivalents.

Total flavonoid content (TFC) estimation

TFC of GDEE was determined according to [29]. Quercetin was used as a standard compound. The absorbance was measured by UV–VIS spectrophotometer at 510 nm, the then the TFC was calculated from the standard calibration curve and was expressed as mg/g equivalent quercetin.

HPLC quantification of vitexin

Vitexin was selected for GDEE standardization as it was recently isolated with large quantities from GDEE [10] in addition to its well documented role in DM management [30, 31].

Sample preparation

GDEE (0.2 g) was dissolved IN 1 mL DMSO. The mixture was then homogenized using ultrasonic bath for 15 min, and filtered through membrane filter (0.45 µm), then 10 µl was injected for analysis into the HPLC system.

Authentic preparation

Serial dilutions of authentic vitexin (99% purity) (10–50 µg/mL) were prepared from stock solution (1 mg/mL). Standard calibration curve was plotted. The calibration curve was fitted by least-squares regression using $y = 11091x + 17{,}222$ as the weighting factor of the peak area [32].

HPLC conditions

Agilent Series 1200 liquid chromatography (Agilent Technologies Inc.) was used for the HPLC analysis. Quaternary

pump, auto-sampler, vacuum degasser, and photodiode array detector were used. Grace Alltima C_{18} analytical column was equipped (5 µm, 250 mm × 4.6 mm, Grace-Alltech) with a flow rate of 1 mL/min at 35 °C. Binary gradient elution system composed of 0.1% phosphoric acid in H_2O and CH_3OH was applied (0–10 min, 25–35% methanol; 10–30 min, 35–45% methanol; 30–45 min, 45–80% methanol). The UV absorption was measured at λ 340 nm. Results were expressed as the mean of three determinations.

Toxicological studies

Animals

Adult male albino rats (130–150 g.b.wt Sprague Dawely strain) were used. The rats were obtained from National Research Center, Dokki, Giza, Egypt animal house colony. They were housed at 55 ± 5% humidity in standard metal cages, in an air-conditioned room (22 ± 3˚C) and provided with standard laboratory diet (vitamin mixture 1%, sucrose 0.2%, mineral mixture 4%, casein (95% pure) 10.5%, corn oil 10%, starch 54.3%). Faculty of Pharmacy, Cairo University Ethics Committee approved this study (No. MP. 1519).

Chemicals and kits

Serum alanine amino-transferase (ALT), aspartate amino-transferase (AST), (Spectrum Diagnostics, Cairo, Egypt), urea and creatinine (Biomérieux-France), glucose (Biomérieux-France), total cholesterol and triglycerides (TG) (Biolabo-France) were colorimetrically estimated using commercial kits.

Alloxan (Sigma Company, USA) and metformin (Chemical Industries Development-CID Co., Egypt) were used for diabetes induction and as antidiabetic standard drugs, respectively.

Acute toxicity

Oral acute toxicity was evaluated according to the Organization for Economic Co-operation and Development, Guideline-423 OECD [33]. The toxicological effects were observed in terms of mortality and expressed as LD50.

Chronic toxicity

Twelve rats were divided into two groups each of 6 rats each. The 1st group kept as control (received daily oral dose of 1 mL saline). The 2nd group received 100 mg/kg b.wt. of GDEE for eight weeks. The blood samples were collected from the *retro-orbital* venous plexus at 0 time and every four weeks. Serum was isolated by centrifugation and subjected for AST, ALT, urea, creatinine, glucose, total cholesterol and triglycerides analysis [34, 35].

In vivo antidiabetic activity

Rats were injected with alloxan (150 mg/kg b.wt.) intraperitoneally to induce DM according to the method described by [36]. The blood glucose level was measured after 72 h, two- and four-weeks intervals. Rats were divided into four groups: 1st group: normal rats that served as normal control, 2nd group: diabetic rats that served as positive control, 3rd and 4th groups: Diabetic rats that received 100 mg/kg b.wt. of GDEE (treated group) and metformin drug as reference drug respectively. After 4 weeks, blood samples were collected from the anaesthetized rats from the *retro-orbital* venous plexus through the eye canthus after an overnight fasting. The blood glucose level was measured. in the serum isolated by centrifugation.

Statistical analysis

The data are presented as mean ± standard error of the mean and were analyzed using paired Student t- test.All data were subjected to statistical analysis using Statgraphics Centurion software package (version 16.1.11, StatPoint Technologies Inc., Warrenton, VA, USA). A probability of less than 0.05 was used as criterion for statistical significance.

Ethics

The study was approved by Faculty of Pharmacy, Cairo University Ethics Committee for Animal Experimentation (No. MP. 1519).

Results

Acute toxicological study of GDEE revealed that its LD_{50} was up to 5.8 g/kg b.wt. No visible signs of toxicity such as general behavioral changes e.g. sniffing, head down, crawling under or over the partner, spasms in both rear legs and convulsions were observed during 24 h of GDEE administration.

Administration of a daily oral dose of GDEE (100 mg/kg b.wt) for eight weeks did not cause any significant effect on AST, ALT, urea, creatinine, cholesterol and triglycerides levels in their sera as compared to the control group Table 1 and Fig. 1a. Meanwhile, the same dose showed a significant decrease in normal blood glucose levels. These results revealed safety upon long term use of GDEE.

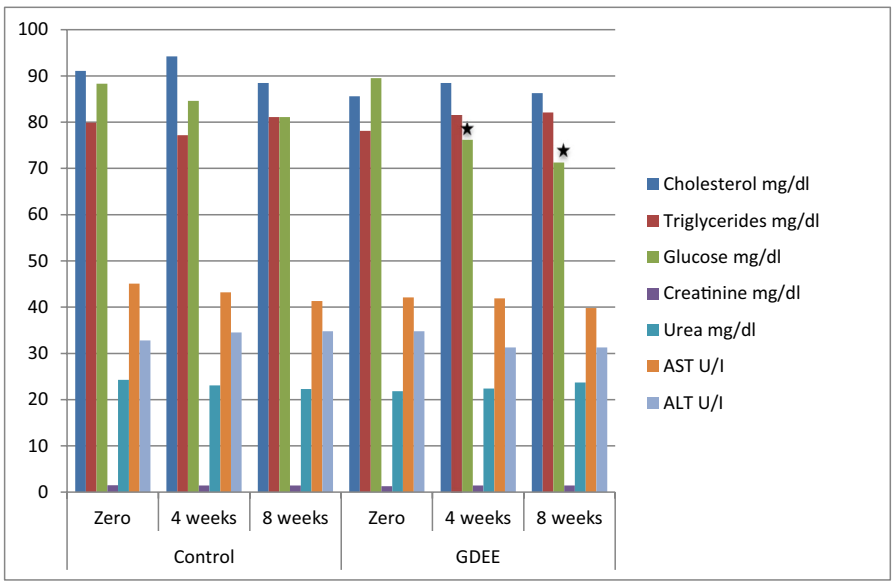
The oral daily dose (100 mg/kg.b.wt.) of GDEE and metformin drug in diabetic male albino rats for one month

Table 1 Effect of long-term administration of GDEE on triglycerides, cholesterol, blood glucose, blood urea, creatinine, and liver enzymes (ALT & AST)

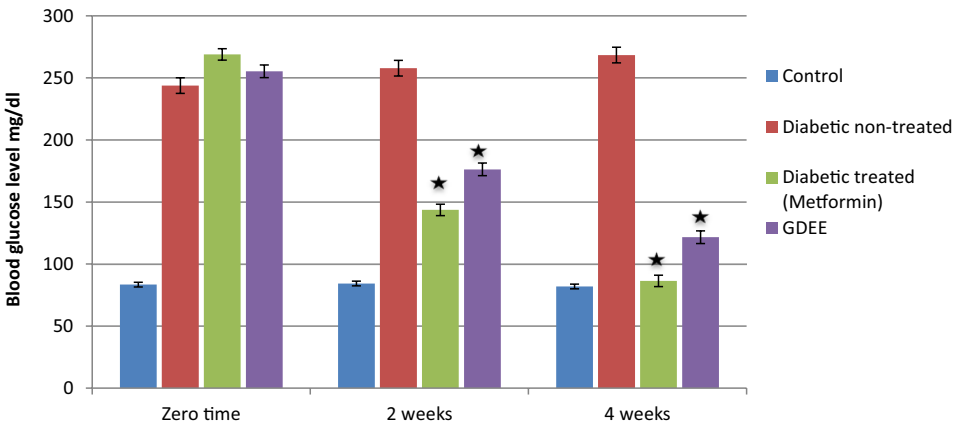
Group	Time weeks	Biochemical changes of serum level						
		Cholesterol mg/dl	Triglycerides	Glucose	Creatinine	Urea	AST U/I	ALT
Control	Zero	91.1 ± 3.5	79.9 ± 2.4	88.3 ± 1.9	1.46 ± 0.03	24.3 ± 0.3	45.1 ± 1.5	32.8 ± 1.2
	4	94.2 ± 2.6	77.2 ± 2.1	84.6 ± 1.7	1.42 ± 0.02	23.1 ± 0.7	43.2 ± 1.3	34.5 ± 1.3
	8	88.5 ± 3.3	81.1 ± 2.3	81.1 ± 1.4	1.45 ± 0.01	22.3 ± 0.3	41.3 ± 1.8	34.8 ± 1.5
GDEE (100 mg/kg b.wt.)	Zero	85.6 ± 2.4	78.1 ± 1.2	89.5 ± 3.1	1.30 ± 0.01	21.8 ± 0.9	42.1 ± 1.2	34.8 ± 1.1
	4	88.5 ± 2.1	81.5 ± 2.9	76.2 ± 1.1*	1.41 ± 0.04	22.4 ± 0.8	41.9 ± 1.4	31.3 ± 1.3
	8	86.3 ± 2.2	82.1 ± 2.2	71.3 ± 1.3*	1.45 ± 0.02	23.7 ± 0.6	39.8 ± 1.4	31.3 ± 1.2

GDEE, *G. decandrus* ethanol extract

* Statistically different from zero time at $p < 0.05$



(a)



(b)

Fig. 1 Effect of administration of 100 mg/kg.b.wt of GDEE on: **a**) Normal rats' cholesterol, triglycerides, blood glucose, creatinine, blood urea and liver enzymes (AST and ALT) serum levels, *: Statistically different from zero time at $p < 0.05$, **b**) Diabetic rats' blood glucose level, *: Statistically different from control group at $p < 0.01$

Table 2 Effect of GDEE administration on level of blood glucose

Groups	Zero time	2 weeks		4 weeks	
	Mean ± S.E	Mean ± S.E	% of change	Mean ± S.E	% of change
Control	83.4 ± 2.1	84.3 ± 1.9	–	81.9 ± 2.2	–
Diabetic non-treated	243.8 ± 6.1	257.8 ± 6.4		268.4 ± 6.3	
GDEE (100 mg/kg)	255.3 ± 5.9	176.3 ± 4.6*	30.9	121.6 ± 3.8*	52.4
Metformin (100 mg/kg)	268.9 ± 7.2	143.6 ± 5.1*	46.6	86.4 ± 2.6*	67.9

GDEE, *G. decandrus* ethanol extract

*Statistically significant different from control group at $p < 0.01$

showed significant change in blood glucose level after 2 weeks with 30.9% and 46.6% decrease, respectively compared with control Table 2 and demonstrated in Fig. 1b. Four weeks administration resulted in continuation in blood glucose reduction by 52.4% and 67.9% for GDEE and metformin respectively.

The total phenolics and flavonoids of GDEE were estimated. They amounted 127.2 ± 0.23 (mg/g equivalent gallic acid) and 85.5 ± 0.21 (mg/g equivalent quercetin) respectively.

Standard calibration curve of vitexin is represented in Fig. 2. The concentration of vitexin in GDEE was 140.70 mg/100gm.

Discussions

Natural compounds are feasible alternatives for diabetes treatment and reinforcements to currently used treatments. They may also reduce the risk of the disease. There are a large number of plants and natural biomolecules exhibited antidiabetic effects. More than one thousand plant species being used for the treatment of type two diabetes mellitus worldwide [37–39]

In the present study, standardized *G. decandrus* crude extract was proved to exhibit promising antidiabetic potential. In 2020, our group evaluated the in vitro antidiabetic potential of *G. decandrus* by screening its crude extract and major isolated phytochemicals for their alpha-glucosidase and *Coxsackie B4* inhibitory activities [10]. The crude extract and the isolated vitexin, protocatechuic acid and quercetin showed promising in vitro antidiabetic potentials.

According to Hodge and Sterner toxicity scale [40] GDEE is in the practically non-toxic category. Also, no signs of chronic toxicity were observed during/after long term oral administration of GDEE Table 1 and Fig. 1a. Moreover, Flavonoids exert their antidiabetic potential through improving insulin secretion, promoting pancreatic β-cells proliferation, reducing β-cells apoptosis, reducing insulin resistance, inflammation and oxidative stress [41].

Phenolic compounds of natural origin can help in preventing or reducing diabetes, obesity, and cardiovascular diseases due to their antioxidant potentials. Additionally, the phenolic compounds ability to inhibit digestive enzymes, including α-glucosidase, lipase and α-amylase, was considered as an effective strategy for type 2 DM management [42]

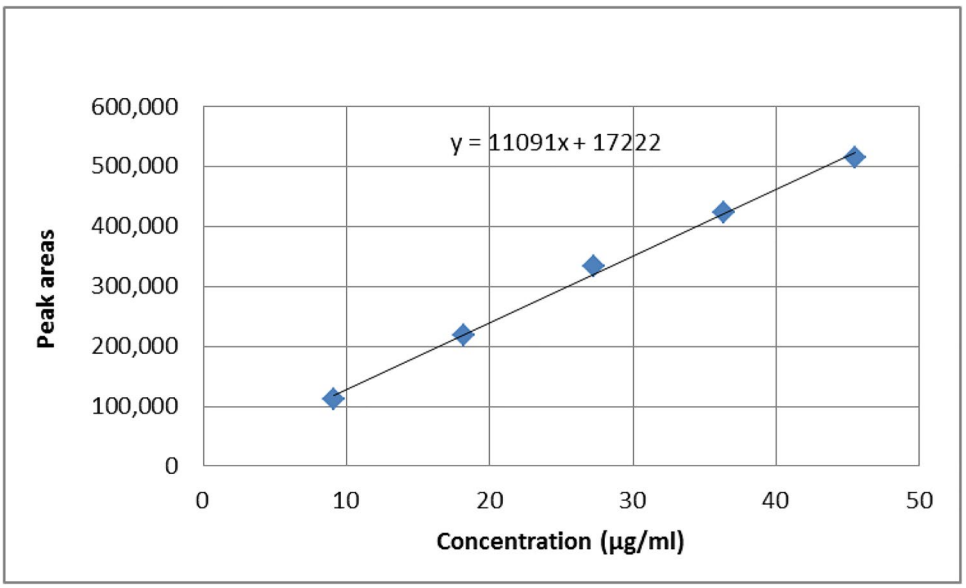


Fig. 2 Standard calibration curve of vitexin for GDEE standardization

The herein reported high flavonoids and phenolic content of *G. decandrus* besides the previously identified and isolated apigenin, luteolin, epiafzelechin, catechins, epicatechins, daphnoretin, protocatechuic acid, vitexin and quercetin [10, 14–17] which exhibited in vitro antidiabetic potential [11, 18–20] are responsible for the potency of the tested crude extract for DM management.

Long term exposure to high levels of blood glucose has been involved in reactive oxygen species (ROS) overproduction of lead to impaired insulin secretion and insulin signaling [43–45]. Thus, high content of phenolics and flavonoids, of well-documented antioxidant potential [46, 47], determined in GDEE is very beneficial in protection against the over production of ROS associated with high blood glucose levels in DM patients. The major limitation of our study was lack of histological evaluations which is an important part of toxicological evaluations; thus, detailed histological assessment including tissue sampling from the internal organs is highly recommended to ensure the safety of the GDEE extract for in vivo administration.

Vitexin is a naturally-occurring flavonoid. It's one of best-known *C*-glycosides due to its pronounced pharmacological activities. It possess antioxidant, α-amylase and α-glucosidase inhibitory effects that can reduce postprandial hyperglycemia and diabetic complications [30, 48]. Vitexin was previously isolated from GDEE [10], thus it was used in our study as marker compound for GDEE standardization.

Conclusion

The present study has successfully proved the safety and the in vivo antidiabetic effect of *G. decancrus* in alloxan induced diabetic model. Our findings presented *G. decancrus* as a promising natural candidate for diabetes management. In the future, detailed histological study to ensure its safety after in vivo administration is essential followed by its formulations in a suitable dosage form for clinical studies.

Declarations

Not applicable.

Conflict of interest No conflict of interest.

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