Antidiabetic activity, alpha-amylase, and alpha-glucosidase inhibitory effect of *Tradescantia spathacea* Swartz extract

Nagaraju Jesetti¹,², Rajasekhar Reddy Alavala³, Subhakar Raju Rapaka³, Umasankar Kulandaivelu³, Koteswara Rao GSN³

¹Research scholar, K L College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh-522502, India
²Department of Pharmacology, Balaji Institute of Pharmaceutical Sciences, Laknepally, Narsampet, Warangal, Telangana-506331, India
³Department of Pharmacy, K L College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh-522 502, India

**Abstract**

*Tradescantia spathacea* Swartz belongs to the genus Commelinaceae, a tropical tree used in many countries as an herbal drug for the care of diabetic patients. The aim of this study was to examine anti-diabetic activity of the *Tradescantia spathacea* Swartz methanolic extract (METSW) and the in-vitro activity of α-amylase, and α-glucosidase was carried out. METSW compared with acarbose inhibition of the α-amylase and α-glucosidase enzyme, METSW exhibited IC₅₀ less than 100 μg/mL would be considered as healthy. The METSW showed IC₅₀ 66.22 ± 0.52 μg/mL α-amylase activity, acarbose revealed an IC₅₀ of 83.25 ± 1.28 μg/mL. METSW demonstrated IC₅₀ levels of 85.37 ± 0.72 μg/mL (y = 0.095x+41.89) inhibition of the α-Glucosidase enzymes. METSW at 400 mg/kg greatly decreased the region under the blood glucose level curve in a typical rat test for oral glucose tolerance. The single dose of the extract decreased dramatically from 211 mg/dl to 89.22 mg/dl at 400 mg/kg METSW in the alloxan induced diabetic model. METSW possesses strong antidiabetic activity in vivo and in vitro. Besides, the extract has also been shown to have a significant inhibitory activity of α-amylase and α-glucosidase which may lead to its anti-hyperglycemic function when used in diabetic patients.

**Introduction**

Diabetes mellitus is a severe chronic autoimmune condition that is stated as a significant source of illness worldwide (Dey et al., 2002). According to projections by the World Health Organization (WHO), the incidence of diabetes is projected to grow by the end of 2020 by 35 percent. Currently, there are over 150 million diabetics worldwide and this is projected to rise to 422 million or more by 2025. Indian statistical prediction suggests an increase in the number of diabetics from 57 million in 2025, the world’s highest diabetics (WHO, 2021). Reasons for this rise include increased sedentary lifestyle, energy-rich food consumption, obesity, longer life
span, etc. Assessment of plant products for the treatment of diabetes mellitus is of growing interest, as they contain many therapeutically potential bioactive substances. Though a significant number of medicinal plants have already been evaluated for their antidiabetic potency, many other medicinal plants still need to be researched in India.

Tradescantia spathacea Swartz (TSW), the Commelinaceae family (synonyms: Discolor rohoeo L and Rhoeospathacea Swartz) (Garcia et al., 1971; Tirumala et al., 2018). It is known in India and Southeast Mexico as “Purple Maguey” (Maguey Morado), and the decoction of the leaves is freely eaten every day as a cancer remedy without current scientific evidence of such property (Garcia et al., 1971). The aqueous form of TSW blocks the antiadrenergic activity (Tirumala et al., 2018). Researchers found proof that contraceptives in rats (Sumalatha et al., 2018). TSW extracts have been used in cosmetics to improve skin appearance (Idaka et al., 1987). Some phytochemicals found in coumarin and steroid compounds of TSW flavonoids, anthocyanins, saponins, carotenoids, waxes, terpenoids (González-Avila et al., 2003). In comparison, the crude methanolic extract TSW, which was tested in vitro, displayed antioxidative activity (González-Avila et al., 2003) and antimicrobial properties (Sumalatha et al., 2018; González-Avila et al., 2003). Due to the lack of in vivo scientific reports which corroborate the antidiabetic development property of TSW, the value of this species exploration is notable.

MATERIALS AND METHODS

Plant extraction

The Tradescantia spathacea Swartz (TSW) plant was collected in the month of July 2018 from the medicinal gardens of Balaji Institute of Pharmaceutical Sciences, Laknepally, Warangal, Telangana, India. Professor Rana Kausar, Department has described the plant specimen. Department of Botany, University of Osmania, Hyderabad, State of Telangana. Powdered the Entire plant in an electronic grinder. The coarse powder was subjected to methanolic extraction (METSW) in Soxhlet’s apparatus and was used for roughly five cycles. The resulting liquid is then washed, dried, and processed in a desiccator for further processing.

Chemicals and reagents

From Sisco Research Laboratories Pvt Ltd, we had bought alloxan, glibenclamide. Blood was harvested for the determination of glucose by snipping the tail with a sharp razor. The blood glucose levels were determined using M/s Boehringer Mannheim, India Ltd. Haemo-Glucoast test (20-800R) glucose strips. All chemicals and reagents that were used in the study were of good quality.

Preliminary phytochemical screening

Diverse phytoconstituents such as alkaloids, flavonoids, steroids, tannins, glycosides, triterpenoids, and saponins were screened for TSW (Roy et al., 2010).

Animals

Male Wistar rats weighing 150–200 g were collected. For acclimatisation, animals were stored at an average temperature of 25°C and 45-55 %RH, with 12 hr each of the dark and light cycles, and a pelleted diet and water ad libitum was served.

Analysis of impact on activity with α-amylase

The α-amylase process was done using the starch-iodine method (Bhandari et al., 2008; Eom et al., 2012; Sudha et al., 2011). 10 μL of α-amylase solution (0.025 mg / mL) was mixed with a 390 μL phosphate buffer (0.02 M comprising 0.006 M NaCl, pH 7.0) comprising varying concentrations of the extract. After incubation at 37°C for 10 min, 100 μL of starch solution (1 percent) was added and the mixture was re-incubated for 1 h. Then 0.1 liters of 1 percent iodine solution were added, and the absorbance at A502 nm was determined after 5 ml of filtered water was added. α-amylase testing, substratum, and blank determinations were done under the same reaction conditions. The inhibition of enzyme activity was computed as (percent inhibition) = (A-C) X100/(B-C), where A= sample absorbance, B= blank absorption (without α-amylase), and C= absorption control (without starch).

Analysis of impact on activity with α-glucosidase

The α-glucosidase inhibitory activity has been measured as suggested (Sudha et al., 2011; Kumkrai et al., 2015). In brief, a mixture of 75 μl of α-glucosidase (Sigma-Aldrich, USA), 225 μl of 80mM Phosphate buffer pH 7.0, and 10-100 μl of various extract concentrations or α-glucosidase inhibitor, acarbose were incubated for 10 min at 37°C. The absorbance was measured optically by using a 510 nm spectrophotometer. Effects are defined as the concentration at which α-glucosidase development is inhibited by 50 percent (IC50).

Acute toxicity studies

Health adult female rats were split into 3 groups (n=5) and were starving overnight. The rats were administered orally with a reducing dosage level of the METSW extract (5000, 1750 mg/kg) (OECD Guidelines No. TG425), and one group was retained.
as a monitor. The animals were observed daily for 4 hours at an interval of 30 min under behavioral, physiological, and autonomic profile including toxicity and mortality and then periodically for any signs of acute toxicity up to 14 days after 6 h and then 24 hours afterwards.

**Oral glucose tolerance test in normal rats**

The TSW effect on blood glucose levels was primarily tested using the oral glucose test. Standard rats that were fasted for six hours were separated randomly into four different groups (n=6) as follows. Group-1: animals are receiving glucose 2 gm/kg orally; Group-2: animals receiving glibenclamide 0.5 mg/kg orally and glucose solution 2 gm/kg; Group-3: animals are receiving METSW 200 mg/kg orally and glucose solution 2 gm/kg orally; Group-4: animals are receiving METSW 400 mg/kg orally, and glucose solution 2 gm/kg orally (Gebremeskel et al., 2020; de Melo et al., 2010).

**Hypoglycemic test in alloxan-induced diabetic rats**

Overnight fasted rats were given a 150 mg/kg intraperitoneal injection of alloxan monohydrate dissolve in cold 0.85 percent saline solution to instigate type 2 diabetes (Alam et al., 2014). Diabetic induction was tested after three days of alloxan injection. Rats had glucose levels over 200 mg/dl (survived without insulin) and were considered a diabetic rat in type-II (Bukhari et al., 2015) used for the experiment. Rats were randomly divided into five groups, as follows. Group-1: standard control animals getting 2ml/kg 1 per cent oral NaCMC; group-2: alloxan (150 mg/kg) diabetic animals getting 2 ml/kg 1 percent oral NaCMC; group-3: alloxan (150 mg/kg) diabetic animals receiving glibenclamide 0.5 mg/kg orally; group-4: alloxan (150 mg/kg) diabetic animals receiving METSW 200 mg/kg orally in 1 percent NaCMC; group-5: alloxan (150 mg/kg) diabetic animals receiving METSW 400 mg/kg orally in 1 percent NaCMC. Following the single dose of drug administration, blood glucose levels were measured at 0, 1, 2, 4, and 6 h to assess the acute hypoglycemic effect of the extract as shown in the previous study (Parra-Naranjo et al., 2017), the rats were divided into five groups of six rats each.

**Statistical analysis**

Values have been demonstrated as ±SEM mean (n=6). Statistical testing was carried out using ANOVA (one-way variance analysis), followed by the Dunnett test. The use of origin pro Software was found statistically important to p<0.001.

**RESULTS**

**Acute toxicity studies**

Acute oral toxicity tests have shown the harmless nature of neither METSW nor any deep toxic reactions detected at a dosage of 5000 mg/kg b.wt, p.o. was observed. That implicitly pronounces a plant extract safety profile. The high oral lethal dose values of METSW (LD<sub>50</sub>Value > 5000 mg kg<sup>-1</sup> b.wt., p.o.) indicate its low acute toxicity.
Preliminary phytochemical analysis

The existence of alkaloids, flavonoids, glycosides, steroids, triterpenoids, and saponins in METSW was shown by a preliminary phytochemical study.

Analysis of impact on activity with $\alpha$-amylase

In the present investigation, METSW displayed significant concentration-dependent inhibition of the $\alpha$-amylase enzyme. Compared with other extracts tested, METSW exhibited IC$_{50}$ less than 100 $\mu$g/mL will be considered as healthy. The METSW showed IC$_{50}$ 66.22 ± 0.52 $\mu$g/mL enzyme inhibition activity (slope y= 0.299x+30.02). Under identical laboratory conditions (Figure 1), All values were outlined as Mean ± S.E.M (n=6), regular acarbose showed an IC$_{50}$ of 83.25 ± 1.28 $\mu$g/mL (slope y= 0.451x+12.45).

Analysis of impact on activity with $\alpha$-glucosidase

The $\alpha$-Glucosidase enzyme is one of the products of diabetes control drugs. The enzyme requires polysaccharide conversion into monosaccharide, which can be consumed by the intestine. Of the plant extracts analyzed, IC$_{50}$ demonstrated less than 100 $\mu$g/mL. METSW demonstrated IC$_{50}$ levels of 85.37 ± 0.72µg/mL (y= 0.095x+41.89) inhibition of the enzymes. At related laboratory conditions, Acarbose displayed an IC$_{50}$ of 51.00 ± 1.23$\mu$g/mL (y= 0.327x+33.32) (Figure 2). All values were outlined as Mean ± S.E.M (n=6).

Test for oral glucose tolerance in normal rats

Blood sugar levels in glucose-fed rats were measured at different periods at dose 200 mg/kg and 400 mg/kg of METSW. It revealed the findings in Figure 3. Each value represents the mean ± SEM. n =6 number of animals in each group. *P<0.001 vs vehicle control, *P<0.05, **P<0.01, ***P<0.001. Compared to respective METSW treated control groups.

Antidiabetic effect of METSW extract in alloxan diabetic rats

The antihyperglycemic outcome of the extracts on diabetic rats’ blood sugar levels was seen in Figure 4. Each value represents the mean ± SEM. n =6 number of animals in each group. *P<0.001 vs vehicle control, *P<0.05, **P<0.01, ***P<0.001. Compared to respective METSW treated control groups.

The mean blood glucose level in rats treated with METSW decreased from 77.12 mg/dl to 75.74 mg/dl at 200 mg/kg body weight and 79.98 mg/dl to 77.71 mg/dl at 400 mg/kg dosage. METSW or glibenclamide has been shown to have greatly increased resistance to glucose in normal rats. Rats obtained METSW (400 mg/kg) and glibenclamide (2 mg/kg) significantly reduced glucose elevation after 30 min relative to the control group (P < 0.05).

DISCUSSION

Diabetes mellitus is an abnormal metabolic disorder that occurs in hyperglycemia and gradually progresses into micro and macro-vascular complications and becomes a major cause of death. Alpha-amylase is a digestive enzyme attached to the brush membrane at the border of the small intestine. Inhibitors of alpha-amylase play a key role in preventing the release of glucose from the supply of dietary carbohydrates and increasing glucose absorption, leading to lower levels of postprandial plasma glucose and further postprandial mining hyperglycemia (Lebovitz, 1997). The inhibition of glucose output from the carbohydrates in the gut or the ingestion of glucose from the intestine has been studied from several natural sources. $\alpha$-amylase catalyses $\alpha$-1,4-glycosidic links to glucose, glycogen and various oligosaccharides for hydrolysis. $\alpha$-glucosidase also breaks down the disaccharides into basic sugars, which are readily available to enter the intestines.

Hence functional and non-toxic alpha-amylase inhibitors have long been pursued. Traditionally selected herbs available across the Telangana area are stated in this report. The key finding of this research shows that both METSW extracts displayed a strong inhibition of $\alpha$-amylase function. In our research, METSW exhibits potent inhibitory action of alpha-amylase and $\alpha$-glucosidase. The present
study gave results to support the traditional claim of antidiabetic activity in herbs. Consequently, the author continued to expand the study to support antidiabetic activity by acute toxicity trials and on in vivo models.

It is now known that there is a progressive decline in the activity and mass of beta cells that can arise in individuals at high risk of type II diabetes. Glibenclamide specifically stimulates insulin activity and is only successful when insulin is present. It remains to be seen if the antidiabetic activity of METSW, close to that found in glibenclamide, could be due to improved insulin secretion.

In this study, the antidiabetic activity of METSW on stable and alloxan-diabetic rats was tested. In normal rats, an oral glucose tolerance test tested the hypoglycemic effect of the extract. The best fasting time for rat development of an OGTT is a 6-h fast (Andrikopoulos et al., 2008; Doan et al., 2018). In each group, the increase in blood glucose showed a strong oral glucose charge after 30 min. In this research, the antidiabetic drug glibenclamide used as a supportive aid reduces postprandial hyperglycemia by increasing the release of insulin from the β-cell. The dosage for oral administration was selected to be 400 mg/kg based on our preliminary examination. Compared to vehicle control, Tradescantia spathacea Swartz extract and glibenclamide improved glucose tolerance, the antidiabetic activity METSW can be due to flavonoid impact. Flavanoids are known to be the active biological concepts in most hypoglycemic and antidiabetic medicinal plants (Scharp and Marchetti, 2014). In order to isolate the lead compound responsible for the antidiabetic activity and alternative modes of action, the extract should, however, be further subject to the development of bioactivity-guided drugs.

**CONCLUSIONS**

In conclusion, the Tradescantia spathacea Swartz methanolic extract possesses strong antidiabetic activity in vivo. In addition, the extract has also been shown to have a significant inhibitory activity of alpha-amylase and alpha-glucosidase, which, when used in diabetic patients, can contribute to its anti-hyperglycemic function. The findings obtained in this analysis provide empirical evidence for corroborating the use of Tradescantia spathacea Swartz for the conventional treatment of diabetics. In addition, to elucidate the plant’s mechanism of action, molecular experiments and isolation of the active ingredient in the extract are strongly needed.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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