



***In vitro* and *In vivo* antidiabetic activity of the leaves of *Ravenala madagascariensis* Sonn., on alloxan induced diabetic rats**

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Abstract:

Ravenala madagascariensis Sonn., (Strelitziaceae) commonly known as Traveler's palm has been widely used in traditional system of medicine for diabetes. The n-Hexane, ethylacetate, ethanol and aqueous leaf extracts of *Ravenala madagascariensis* were examined at a concentration of 50gram/litre using an *in vitro* method to assess the possible effects on glucose diffusion across the gastrointestinal tract and compared to control conducted in the absence of extracts. The ethanolic and aqueous extracts showed a significant inhibitory effect on glucose diffusion *in vitro* and accordingly were screened at 200 and 400mg/kg, b.w for the *in vivo* antidiabetic activity on alloxan induced diabetic rats. Glibenclamide (10mg/kg, b.w) used as reference standard. Both extracts showed a significant antidiabetic activity. The ethanolic extract was more effective in reducing the blood glucose levels during acute ($p < 0.001$) and prolonged treatment ($p < 0.001$) and were comparable with that of standard thus validating the traditional claim of the plant.

Key words:

Ravenala madagascariensis Sonn., Leaf extracts, Antidiabetic activity, Glucose diffusion method, Alloxan, *in vitro* antidiabetic

Introduction:

Use of plants for human health care is as ancient as human beings themselves. Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, resulting in hyperglycaemia leading to acute and chronic complications [1]. The incidence of diabetes mellitus is on rise all over the world. Synthetic drugs are likely to give serious side effects in addition they are not suitable for intake during conditions like pregnancy [2-4]. Hence, search for a new drug with low cost, more potential, without adverse effects is being pursued in several laboratories all around the world.

Ravenala madagascariensis Sonn., (Strelitziaceae) commonly called as Traveler's tree, is a native of Madagascar, often found cultivated in Indian gardens. It is a palm like tree, with simple alternate leaves forming a fan like crown. It is widely used in folklore medicine in the treatment of diabetes and kidney stone problems [5,6]. The seeds were reported to be antiseptic [7]. The present paper reports the *in vitro* and *in vivo* antidiabetic activity of the leaves of *Ravenala madagascariensis* Sonn.

Materials and Methods:

Plant material

The fresh leaves of *Ravenala madagascariensis* Sonn., (Strelitziaceae) were collected from Subramaniapuram Park, Trichy District, TamilNadu, during June 2009 and authenticated by Botanical survey of India, Coimbatore, No. BSI / SC /5/ 23 / 09-10 / Tech-622. A voucher specimen was deposited in the Department of Pharmacognosy, Madras Medical College for future reference.

Experimental animals

Healthy Wistar Albino rats of either sex, weighing about 150-200g were used for the study. All processes were approved by the Institutional Animal Ethical Committee which is certified by the Committee for the purpose of control and supervision of experiments on animals, India (CPCSEA); Reg. No 243. The animals were kept in clean and dry polycarbonate cages and maintained in a well ventilated animal house with 12 hour light – 12 hour dark cycle. The animals were fed with standard pellet diet and water was given as libitum. For experimental purpose, the animals were kept fasting overnight but allowed for access to water.

Chemicals

Alloxan was obtained from S.D. fine chemicals limited, Mumbai. Glibenclamide was purchased from Aventis Pharmaceuticals limited, Goa. All solvents used were of analytical grade obtained from E. Merck, Mumbai, India.

Preparation of extracts

The shade dried powdered leaves were subjected to successive extraction using n-hexane, Ethyl acetate, Ethanol by continuous percolation process in soxhlet apparatus. The aqueous extract was prepared by the maceration with water. Each extract was concentrated by distilling off the solvent and evaporated to dryness. The extracts were dissolved in 1% carboxy methyl cellulose (CMC) and used for the present study.

***In vitro* antidiabetic activity (By Glucose Diffusion Inhibitory Study) [8]**

A simple model system was used to evaluate the effects of plant extracts on glucose movement *in vitro*. The model was adapted from a method described by Edwards *et al.*, [9] which involved the use of a sealed dialysis tube into which 15ml of a solution of glucose and sodium chloride (0.15M) was introduced and the appearance of glucose in the external solution was measured. The model used in the present experiment consisted of a dialysis tube (6cmX15mm) into which 1ml of 50g/litre plant extract in 1% CMC and 1ml of 0.15M sodium chloride containing 0.22M D-glucose was added. The dialysis tube was sealed at each end placed in a 50ml centrifuge tube containing 45ml of 0.15M sodium chloride. The tubes were placed on an orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored at set time intervals.

Acute Toxicity Study [10]

Acute oral toxicity study was carried out for ethanolic and aqueous extracts using Acute

toxic class method described as per OECD guidelines – 423.

***In vivo* antidiabetic activity**

Experimental Induction of Diabetes [11]

The rats are injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 120 mg/kg body weight. 2 weeks after treatment, rats with blood glucose level above 300 mg/dl were selected for the study.

Experimental Design

The animals were randomly divided into 7 groups of six animals each after the induction of diabetes.

Group 1: Non diabetic control rats received 1% CMC, 2ml/kg body weight per oral, once daily for 5 weeks.

Group 2: Diabetic control rats received 1% CMC, 2ml/kg body weight per oral, once daily for 5 weeks.

Group 3: Diabetic rats received standard drug Glibenclamide, 10 mg/kg body weight, in 1% CMC, orally, once daily for 5 weeks.

Group 4: Diabetic rats given ethanolic leaf extract, 200 mg/kg body weight of *Ravenala madagascariensis* Sonn., made a fine suspension with 1% CMC, orally for 5 weeks.

Group 5: Diabetic rats given ethanolic leaf extract, 400 mg/kg body weight of *Ravenala madagascariensis* Sonn., made a fine suspension with 1% CMC, orally for 5 weeks.

Group 6: Diabetic rats received aqueous leaf extract, 200 mg/kg body weight of *Ravenala madagascariensis* Sonn., made a fine suspension with 1% CMC, given orally for 5 weeks.

Group 7: Diabetic rats received aqueous leaf extract, 400 mg/kg body weight of *Ravenala madagascariensis* Sonn., made a fine suspension with 1% CMC, given orally for 5 weeks.

The blood samples were collected from the retro orbital plexus at 1, 3, 5, 7, 24hrs (Acute study) and at the end of 1, 3 and 5

Table 1: Effect of various plant extracts (50g/litre) on the movement of glucose out of dialysis tube over 27hr incubation period

Extract	1h	3h	5h	24h	27h
Control (in the absence of extract)	134.33± 1.20	201.33± 1.76	244.33± 1.76	312.33± 2.02	316.66± 1.76
n-Hexane extract (50g/l)	105.33± 1.20***	184± 1.15***	216± 1.15***	293± 1.15***	303.33± 1.20***
Ethylacetate extract (50g/l)	94± 1.15***	155.33± 0.88***	193.66± 0.88***	256.33± 1.45***	262.33± 0.88***
Ethanol extract (50g/l)	76.33 ±0.88***	105± 1.73***	143.66± 1.20***	203± 1.52***	208± 1.15***
Aqueous extract (50g/l)	83.33± 0.88***	116.33± 0.88***	157.33± 1.20***	228.66± 1.76***	232± 2.18***

Values are expressed as mean ± SEM n=3; Data were analysed using one way ANOVA followed by Tukey-Kramer multiple comparison test; ***p<0.001 compared to control.

weeks (Chronic study). Blood glucose levels were determined by the glucose oxidase method.

Statistical Analysis

Data are expressed as mean ± S.E.M. Statistical comparison between groups were done by one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test to analyze the differences. p<0.001 were considered as significant.

Results:

Effects of Various Extracts on Inhibitory Glucose Diffusion:

The effect of various extracts on glucose diffusion inhibition was depicted in Fig 1. At the end of 27 hrs, glucose movement of control (without plant extract) in the external solution had reached a plateau with a mean glucose concentration above 300mg/dl (316.66±1.76). It was evident from the graph that the ethanol and aqueous extracts were found to be potent inhibitors of glucose diffusion (p<0.001) compared to control. The ethanol extract was found to be more potent than other extracts showing the lowest mean glucose concentration of 208±1.15 mg/dl at the end of 27 hrs (Table.

1). Thus the ethanol and aqueous extracts were selected for further *in vivo* studies.

Acute Toxicity Study

On the basis of toxicity study, it was observed that the ethanolic and aqueous extracts were nontoxic and did not induce death at the highest single dose, 2000 mg/kg b.w. per oral. No toxic symptoms like behavioral changes, locomotion, convulsions etc., were observed.

In vivo antidiabetic activity

Acute study

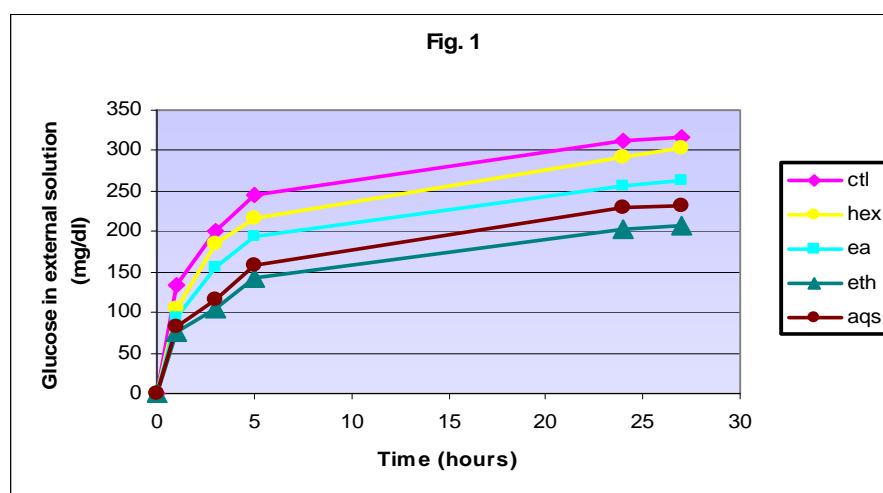
The antidiabetic effect of ethanolic and aqueous extracts at the dose of 200 and 400 mg/kg b.w. on alloxan induced diabetic rats is given in Table.2

Single oral administration of both ethanolic and aqueous extracts produced a dose dependent hypoglycemia on alloxan induced diabetic rats. It is clear from the data that the blood glucose levels of control diabetic animals continued to increase whereas extract treated diabetic rats showed significantly reduced glucose levels. The ethanolic extract showed a significant antidiabetic activity, whereas aqueous extract showed a slightly lower effect in a dose dependent manner.

Table 2: Effect of ethanolic and aqueous extracts of the leaves of *Ravenala madagascariensis* Sonn., on blood glucose levels in alloxan induced diabetic rats (mg/dl) - Acute study

Group	0h	1h	3h	5h	7h	24h
Normal	94.5 \pm 0.76	104 \pm 0.81	112.83 \pm 0.60	114.16 \pm 0.54	117.33 \pm 0.33	107.16 \pm 0.60
Diabetic control	357.83 \pm 0.3	363.66 \pm 0.61	367 \pm 0.42	371.5 \pm 0.42	392.83 \pm 0.30	425 \pm 0.57
Diabetic + Glibenclamide (10mg/kg)	334.33 \pm 0.5	324.83 \pm 0.47***	312 \pm 0.81***	304.33 \pm 0.88***	286 \pm 0.57***	262.5 \pm 0.56***
Diabetic + Ethanolic Ext (200mg/kg)	346.83 \pm 0.70	336.5 \pm 0.76***	324.5 \pm 0.42***	316.5 \pm 0.76***	295.5 \pm 0.99***	283.33 \pm 0.49***
Diabetic + Ethanolic Ext (400mg/kg)	382.83 \pm 0.60	355.83 \pm 0.90***	343.66 \pm 0.66***	304.16 \pm 1.01***	293.5 \pm 0.76***	253.33 \pm 0.71***
Diabetic + Aqueous Ext (200mg/kg)	370.66 \pm 0.88	366.33 \pm 0.55	355 \pm 0.57***	323.83 \pm 0.60***	313.5 \pm 0.76***	299.33 \pm 0.76***
Diabetic + Aqueous Ext (400mg/kg)	384.5 \pm 0.76	371.16 \pm 1.42***	352.5 \pm 0.84***	316.16 \pm 1.07***	303.83 \pm 0.83***	294.5 \pm 1.40***

Data were analysed using one way ANOVA followed by Tukey-Kramer multiple comparison test; ***p<0.001 compared to diabetic control

**Fig.1:** Effect of 50g/l of various extracts of *Ravenala madagascariensis* Sonn., on the movement of glucose diffusion out of dialysis tube.

Values are mean of three observations; Values are expressed as mean + SEM n=6

Table 3: Effect of ethanolic and aqueous extracts of the leaves of *Ravenala madagascariensis* Sonn., on blood glucose levels in alloxan induced diabetic rats (mg/dl)- Chronic study

Group	1 week	3 weeks	5 weeks
Normal	103.83±0.47	105.5±0.42	107.83±0.47
Diabetic control	483.16±0.3	514.5±0.56	546.5±0.42
Diabetic + Glibenclamide (10mg/kg b.w.)	190.66±0.66***	150.66±0.61***	111.16±0.90***
Diabetic + Ethanolic Ext (200mg/kg b.w.)	234.66±1.14***	160.66±0.61***	127.16±0.60***
Diabetic + Ethanolic Ext (400mg/kg b.w.)	203.66±0.55***	153.5±0.61***	118.16±0.47***
Diabetic + Aqueous Ext (200mg/kg b.w.)	255.83±0.94***	170.5±0.99***	132.33±0.42***
Diabetic + Aqueous Ext (400mg/kg b.w.)	225.33±1.17***	167±0.63***	122.5±0.56***

Values are expressed as mean + SEM n=6; Data were analysed using one way ANOVA followed by Tukey-Kramer multiple comparison test; ***p<0.001 compared to diabetic control.

Chronic study

Chronic administration of *Ravenala madagascariensis* Sonn., extracts to alloxan induced diabetic rats for 5 weeks produced a significant blood glucose reduction. At the end of 5 weeks, ethanolic extract produced a significant reduction of 79% (p<0.001) at 400mg/kg b.w compared to diabetic control. On the other hand, glibenclamide produced a significant blood glucose reduction of 80% and the results are displayed in Table.3.

Discussion:

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world [12]. There is a steady rise in the rate of incidence of Diabetes mellitus and estimated that 1 in 5 may be diabetic by 2025 [13]. Over 400 plants have been documented as being useful for the control of blood glucose concentration. However the majority of these plants have yet to be scientifically or medically evaluated [14].

Antihyperglycemic activities of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/or to stimulate insulin

secretion [15-21]. In the present study, research has been carried out to evaluate the potential of various extracts to additionally retard the diffusion and movement of glucose in the intestinal tract [22]. But the *in vitro* inhibitory activity cannot be always related to *in vivo* activity [23]. Thus the concept needs to be demonstrated in preclinical animal studies. Accordingly the ethanolic and aqueous extracts which showed the maximum inhibition of glucose diffusion were selected for the *in vivo* study. Our *in vivo* study showed administration of both ethanolic and aqueous leaf extracts of *Ravenala madagascariensis* Sonn., produced a significant reduction in blood glucose level in a dose dependent manner. However the ethanolic extract was found to be more effective than the aqueous extract which may be due to higher solvent extraction capacity of ethanol rather than water. The results of the study strongly suggest that *Ravenala madagascariensis* Sonn is useful in the treatment of Diabetes mellitus. Further studies are needed to isolate the phytoconstituent responsible for the antidiabetic activity.

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