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Antioxidant Activity of Ravenala madagascariensis Sonn. Leaves on Alloxan Induced Diabetic Rats

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Abstract: Our previous studies showed that *Ravenala madagascariensis* Sonn. leaf extract have antidiabetic, hypolipidemic and renoprotective effects on alloxan induced diabetic rats. Reactive oxygen species have been implicated in the mechanism of damage of red blood cells in diabetic patients. Hence the present study was carried out to investigate antioxidant activity of leaf extracts of *Ravenala madagascariensis* against oxidative stress in the RBC's of alloxan induced diabetic rats. Evaluations were made for lipid peroxidation, catalase and reduced glutathione levels on blood. Chronic administration of ethanolic extract (400 mg/kg body weight) showed a significant reduction ($p< 0.001$) in the lipid peroxidation level which were comparable with that of the normal. The reduced levels of GSH content and catalase activity were increased significantly ($p< 0.001$) on treatment with the extract in a dose dependent manner. However the aqueous extract showed a poor antioxidant activity.
Keywords: antioxidant activity, *Ravenala madagascariensis*, catalase, glutathione, lipid peroxidation.

Introduction

Oxidative stress generated by hyperglycemia and hyperlipidaemia is regarded as an important mediator of diabetic complications. The presence of free radicals and the simultaneous decline of antioxidant defense mechanisms were observed in diabetic complications.[1] It may cause oxidative damage of cellular membranes and changes in the structural and functional integrity of subcelluar organelles and may produce effects that result in haemorrhagic disturbances.[2,3] It has been shown that red blood cells (RBCs) from diabetic patients exhibit reduced membrane deformability, increased viscosity, and abnormal adherence to endothelial cells.[4] The rate of production and elimination of free radicals is always balanced by the antioxidant defense mechanism and any shift in this delicate balance will lead to cellular damage. Thus antioxidants have gained importance in recent years due to their ability to neutralize free radicals and their actions.[5]

Ravenala madagascariensis Sonn., was claimed to be widely useful for the treatment of diabetes and kidney stone problems.[6] The antidiabetic, hypolipidemic and renoprotective of activity of *Ravenala*

madagascariensis leaf extract were evaluated and reported in our previous studies.[7,8] Since the reactive oxygen species have been implicated in the mechanism of damage of red blood cells in diabetic patients, the present study was carried out to investigate antioxidant activity of leaf extracts of *Ravenala madagascariensis* against oxidative stress in the RBC's of alloxan induced diabetic rats.

Materials And Methods

Plant Material

The fresh leaves of *Ravenala madagascariensis* Sonn., (Strelitziaceae) were collected from Subramaniapuram Park, Trichy District, TamilNadu 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 (weighing about 150-200g) were procured from Madras Medical College Animal House. The entire process was 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 7/243. 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 SD fine chemicals Limited, Mumbai. Glibenclamide was purchased from Aventis Pharmaceuticals Limited, Goa. All the chemicals used were of analytical grade obtained from E. Merck, Mumbai, India.

Preparation Of Extracts

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

Acute Toxicity Study [9, 10]

Acute oral toxicity study was carried out for the ethanolic and aqueous extracts using acute toxic class method described as per OECD guidelines – 423.

Experimental Induction Of Diabetes [11]

The rats were 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: Normal untreated rats
Group 2: Diabetic control rats
Group 3: Diabetic rats given Glibenclamide 10 mg/kg body weight.
Group 4: Diabetic rats given ethanolic leaf extract of *Ravenala madagascariensis* Sonn., (200 mg/kg body weight)
Group 5: Diabetic rats given ethanolic leaf extract of *Ravenala madagascariensis* Sonn., (400 mg/kg body weight), orally for 5 weeks.

Group 6: Diabetic rats given aqueous leaf extract of *Ravenala madagascariensis* Sonn., (200 mg/kg body weight), orally for 5 weeks.
Group 7: Diabetic rats given aqueous leaf extract of *Ravenala madagascariensis* Sonn., (400 mg/kg body weight), orally for 5 weeks.

Antioxidant Activity[12-16]

For the evaluation of antioxidant activity, blood was collected in heparinized tubes and the plasma was separated by centrifugation at 1200 rpm for 15 minutes. The buffy coat was removed and the erythrocytes were washed three times with physiological saline. Aliquots of erythrocytes were kept at 4 °C until analysis.

Lipid Peroxidation (LPO)

To 2 ml, 5% suspension of separated RBC in 0.1 M phosphate buffered saline, 2 ml of 28% trichloroacetic acid was added and centrifuged. One ml of 1% thiobarbituric acid was added to 4 ml of supernatant, heated in boiling water for 60 min and cooled immediately. The absorbance was measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) ($1.56x\ 10^5$), and expressed in terms of nanomoles of MDA/g Hb.

Catalase (CAT)

Catalase activity was determined in erythrocyte lysate using Aebi's method with some modifications. 50 µl of the lysate was added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1ml of 30 mM H_2O_2. Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1} was used to determine the catalase activity is expressed as micromoles per milligram of protein.

Reduced Glutathione (GSH)

GSH was estimated using previously reported method of Ellman *et al.*, 1959. In this method, 0.02 ml of fresh or citrated blood was added to 9.0 ml of distilled water to which 1.0 ml of phosphate buffer (pH 8.0) was added. 3 ml of this solution was placed into each of two Beckman 1-cm cells, using one to adjust the absorbance to zero. To the other, 0.02 ml, 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB) absorbance was determined at 420 nm after 30 min. Results were expressed as mmol (SH)/l blood. The concentration of reduced glutathione was calculated using the following formulae:

Co = 36.8 X Absorbance

Where, Co - Original concentration
A - Absorbance at 420 nm

Statistical Analysis

Datas were expressed as mean $\pm$ standard error of mean. Statistical comparison between groups were done by one way analysis of variance (ANOVA), followed by Tukey Kramer test to analyze the differences.

Results

The results of antioxidant activity of the ethanolic extracts of leaves of *Ravenala madagascariensis* were tabulated in Table 1. The antioxidant studies revealed that at the end of 5 weeks there was increased level of lipid peroxidation and reduced content of GSH in diabetic control rats. The catalase activity has also been decreased. On the other hand, oral treatment of diabetic rats with ethanolic extract, daily for 5 weeks ameliorated alterations in the lipid peroxidation and antioxidant parameters. Chronic administration of ethanolic extract (400 mg/kg body weight) showed a significant reduction ($p< 0.001$) in the lipid peroxidation level which

were comparable with that of the normal. The reduced levels of GSH content and catalase activity were very well reverted back to normalcy by the ethanolic extract of *Ravenala madagascariensis* Sonn., in a dose dependent manner. However the aqueous extract showed a poor antioxidant activity.

Table. 1 Effect of Ethanolic and Aqueous extract of *Ravenala madagascariensis* Sonn., in the erythrocytes of normal and alloxan induced diabetic rats

Group	Lipid Peroxidation nm MDA/g Hb	GSH mmol (SH)/ l blood	Catalase µm/mg protein
Normal	60.45 ± 2.06	4.25±0.04	182.13±1.05
Diabetic Control	129.36± 2.32	0.83±0.04	113.82±1.795
Glibenclamide (10mg/kgb.w.)	85.84±0.47***	3.87±0.03***	161.86±1.11***
Ethanolic Extract (200mg/kg)	76.59±1.53***	3.47±0.02***	166.59±0.79***
Ethanolic Extract (400mg/kg)	69.24±1.42***	3.85±0.02***	174.62±0.90***
Aqueous Extract (200mg/kg)	100.01±1.59***	1.55±0.02***	134.90±1.05***
Aqueous Extract (400mg/kg)	92.42±1.56***	2.00±0.03***	144.16±1.11***

Datas are expressed as Mean ± SEM of 6 rats
Datas were analyzed using one way ANOVA followed by Tukey-Kramer multiple comparison test
*** P<0.001 compared to diabetic control

Discussion

The involvement of free radicals in diabetes and the role of these in lipid peroxidation and the antioxidant defense system have been studied. Treatment with leaf extracts of *Ravenala madagascariensis* was carried out to measure its antioxidant potential in blood. The results showed increased lipid peroxidation in diabetic rats, and this may be due to the increase in blood glucose levels, with auto-oxidation, generate free radicals. Increased lipid peroxidation in erythrocytes cause decreased cell survival, altered membrane lipid asymmetry, hypercoagulability, increased adhesivity to endothelium [17] and focal occlusion.[18] These levels were decreased significantly in extract treated rats. The results showed that administration of ethanolic extract tend to bring the peroxides back to near normal levels. Also a significant decrease in GSH levels is observed in diabetic rats. GSH plays a vital role in the protection of cells against free radicals. Decreased activity of Glucose-6-Phosphate dehydrogenase and inadequate NADPH lowers the GSH level. The decrease in GSH levels represents increased utilization due to oxidative stress.[19] Chronic administration of *Ravenala madagascariensis* Sonn., resulted in replenishment of GSH maintaining the antioxidant activity.[20,21] Catalase forms the first line of defense against free radicals. Catalase reduce H_2O_2 and thus protect the erythrocyte cells. The Catalase levels clearly shows *Ravenala madagascariensis* contains a free radical scavenging activity, which could exert a beneficial action against pathologic alterations caused by the free radicals. Thus we conclude that after 5 weeks of chronic administration, the ethanolic extract at 400mg/kg b.w was found to be more effective. Further investigations can be putforth in isolating the phytoconstituents responsible for the therapeutic effect.

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