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Evaluation of *Alternanthera sessilis Linn* against Diabetes Induced Retinopathy in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Diabetes Retinopathy (DR) is a major microvascular complication occurs due to the Chronic Diabetes. The objective of present study is to evaluate the *Alternanthera sessilis Linn* against Diabetes Induced Retinopathy in Rats. Diabetes was induced in rats by injecting 120 mg/kg of Alloxan monohydrate intra-peritoneally to overnight-fasted rats (12 h). After 72 h of injection, fasting blood glucose level was measured. Diabetic animals were separated into six groups (n = 6). Group 1 (normal control), received vehicle orally. Group 2 (Diabetic control group), received 1 ml of normal saline. Group 3 (reference group): Diabetics rats, received Glibenclamide (10 mg/kg b w, p. o). Group 4 and 5 (Test group-1 and Test group-2 respectively), received the ethanolic extract of

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Alternanthera Sessilis 500 and 1000 mg/kg bw respectively and the treatment was continued for 42 days. At the end of the study blood was collected from retro orbital plexus for estimation of biochemical parameters and antioxidant parameters. Diabetic rats exhibited the significant increase in blood glucose, total cholesterol, triglycerides, lipid peroxidation, aldose reductase and significant decrease in superoxide dismutase, catalase, reduced glutathione in comparison to normal rats. Diabetic rats treated with ethanolic extract of *Alternanthera Sessilis* at doses of 500 and 1000 mg/kg b w showed significant decrease in blood glucose, total cholesterol, triglycerides, lipid peroxidation, aldose reductase and significant increase in superoxide dismutase, catalase, reduced glutathione in comparison to diabetic rats in dose dependent manner.

Keywords: Alternanthera Sessilis; diabetic retinopathy; diabetic parameters and oxidative parameters.

1. INTRODUCTION

"Diabetic retinopathy (DR) is a clinical pathology that arises due to diabetic mellitus and uncontrolled hyperglycemic conditions. The most common clinical signs include visual impairment due to changes in the microvascular structure, lesions in the retina due to the presence of hard or soft exudates, microaneurysms, thickening, and fluid buildup in the retina" [1]. "There are two main stages of diabetic eye disease. NPDR (non-proliferative diabetic retinopathy). This is the early stage of diabetic eye disease. Many people with diabetes have it. PDR (proliferative diabetic retinopathy) PDR is the more advanced stage of diabetic eye disease. It happens when the retina starts growing new blood vessels. This is called neovascularization" [2]. "These fragile new vessels often bleed into the vitreous. If they only bleed a little, you might see a few dark floaters. If they bleed a lot, it might block all vision. As per International Diabetes Federation, there will be approximately 629 million adults will suffer with a diabetic condition by the year 2045 [3], even though the international predominance rate for DR is about 93 million" [4,5,6]. Hence, "DR has raised community apprehension throughout the world. Early finding is the best anticipatory assess for an exacerbation of DR" [7]. The presently available interventions in treating DR, such as antivascular endothelial growth factor, panretinal laser photocoagulation (PRP), vitrectomy, and corticosteroid injections, are often accompanied by obligatory side effects. In this connection the present study is conducted to evaluate the *Alternanthera sessilis* against diabetes induced retinopathy in rats.

2. MATERIALS AND METHODS

2.1 Plant Collection

Alternanthera sessilis plant is collected from surroundings of Anantapur district, Andhra Pradesh and authenticated by Dr. Madhava Chetty, Assistant Professor, SVU, Tirupathi, Andhra Pradesh, India.

2.2 Extraction

The collected leaves of *Alternanthera sessilis* were shade dried and powdered and subjected to maceration with ethanol (EEAS). Powder is placed inside a container, the menstruum (ethanol) is poured on top until completely covered the powder material. The container is closed and kept for four days. The container is stirred periodically and fifth day the micelle is separated from marc by decantation and filtered. The solvent is evaporated by using rota evaporator.

2.3 Phytochemical Investigation

Ethanolic extract of *Alternanthera sessilis* is subjected to presence of phytoconstituents.

2.4 Drugs and Chemicals

Alloxan was purchased from sigma-Aldrich and various biochemical parameters were estimated by using commercially available erba kits and other chemicals used were of analytical grade.

2.5 Experimental Animals

Male wistar rats, weigh between 180 and 200g were procured from Raghavendra Enterprises, Bangalore, India. All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethical Committee, Protocol No. IAEC/XV/03/RIPER/2020. All animals were maintained under adequate conditions at an ambient temperature of 21±2^{o} c, and were subjected to 12 h light and dark cycle. They were fed with standard pellet diet and water ad libitum.

Animals were kept for 7 days in laboratory for habituation.

2.6 Induction of Diabetes and Experimental Design

Diabetes was induced in rats by injecting 120 mg/kg of Alloxan monohydrate intra-peritoneally dissolved in 0.9% w/v cold normal saline to overnight-fasted rats (12 h). After 72 h of injection, fasting blood glucose level was measured. The animals that did not develop more than 200 mg/dL glucose levels were omitted from the study. Diabetic animals were divided into six groups (n = 6). Group 1 (normal control), received the vehicle orally. Group 2 (Diabetic control group), received 1 ml of normal saline. Group 3 (reference group): Diabetics rats, received Glibenclamide (10 mg/kg b w, p. o). Group 4 and 5 (Test group-1 and Test group-2 respectively), received ethanolic extract of Alternanthera Sessilis at doses of 500 and 1000 mg/kg bw respectively and the treatment was persistent for 42 days. At the end of the study blood was collected from retro orbital plexus for estimation of biochemical parameters and antioxidant parameters.

3. STATISTICAL ANALYSIS

Graph pad prism 2.6 was used to analyze the data and results were expressed as mean ± sem One-way ANOVA and the post hoc Tukey test were used to analyze the significant differences among groups. A probability of $p < 0.05$ was defined as a statistically significant result.

4. RESULTS

4.1 Effect of Ethanolic Extract of *Alternanthera sessilis* on Body Weight

Body weight of all rats were measured on day 1 (Initial body weight) and final day of study (Final body weight) and change in body weight.

Table 1. Effect of ethanolic extract of *Alternanthera sessilis* on body weight

Group	Initial body weight	Final body weight	Weight gain (%)
Normal control	172 ± 2.64	198 ± 0.45	26.2±2.98
Diabetic control	175 ± 2.37	149 ± 2.92***	-26±2.45
Diabetic + EEAS (500mg/kg)	176±1.90	196 ± 1.37###	20±1.59
Diabetic+ EEAS (1000mg/kg)	175 ± 2.07	204 ± 2.27###	29±2.09
Diabetic + Glibenclamide (10mg/kg)	175 ± 2.93	205 ± 2.76###	30±4.77

*Values are expressed as mean ± SEM (n=6 rats in each group), ***P<0.001, compared to normal control, ###P<0.001 compared to diabetic control*

Table 2. Effect of ethanolic extract of *Alternanthera sessilis* on blood glucose and lipid parameters

Group	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)
Normal control	104±4.41	79.3 ± 3.63	77.2 ± 4.38	30.4 ± 1.32
Diabetic control	296±9.91	174 ± 7.80	175 ± 2.0	18 ± 0.62
Diabetic + EEAS (500mg/kg)	183±3.46***	127 ± 2.16***	116 ± 5.44***	21.5 ± 0.83***
Diabetic + EEAS (1000mg/kg)	112±6.93###	96.4 ± 1.43###	80.9 ± 6.36###	28.7 ± 0.87###
Diabetic + Glibenclamide (10mg/kg)	92.8±4.24###	86.3 ± 3.89###	70.8 ± 2.31###	27.1 ± 0.71###

*Values are expressed as mean ±SEM (n=6 rats in each group), ***P<0.001, compared to normal control, ###P<0.001 compared to diabetic control.*

Table 3. Effect of ethanolic extract of *Alternanthera sessilis* on oxidative parameters

Group	Superoxide dismutase (U/mg protein)	Catalase (µmol of H_2o_2 decomposed/min/mg protein)	Reduced glutathione (µg/g tissue)	Lipid peroxidation (µ mol MDA/ mg protein)
Normal control	33±1.12	67.8 ± 2.49	80.3±0.74	17.1±0.89
Diabetic control	15.4±0.85***	31 ± 3.15***	32.3±1.14***	69.2±1.75***
Diabetic + EEAS (500mg/kg)	23.6±0.76###	51.9 ± 2.01###	55.7±0.70###	23.4±1.29###
Diabetic + EEAS (1000mg/kg)	30.1±2.00###	53.9 ± 3.13###	77.7±0.70###	16.2±0.85###
Diabetic + Glibenclamide (10mg/kg)	33.9 ± 0.85###	60.3± 0.96###	80.1±0.89###	17±0.68###

*Values are expressed as mean ± SEM (n=6 rats in each group), ***P<0.001, compared to normal control, ###P<0.001 compared to diabetic control.*

Table 4. Effect of ethanolic extract of *Alternanthera sessilis* on Aldose reductase

Group	Aldose reductase (U/mg lens wet weight)
Normal control	2.12±1.12
Diabetic control	3.4±0.85***
Diabetic + EEAS (500mg/kg)	2.6±0.76###
Diabetic + EEAS (1000mg/kg)	2.4±2.00###
Diabetic + Glibenclamide (10mg/kg)	2.1 ± 0.85###

*Values are expressed as mean ± SEM (n=6 rats in each group), ***P<0.001, compared to normal control, ###P<0.001 compared to diabetic control.*

4.2 Effect of ethanolic extract of *Alternanthera sessilis* on blood glucose and lipid parameters

At the end of the study blood glucose and lipid profile was measured.

4.3 Effect of ethanolic extract of *Alternanthera sessilis* on oxidative parameters

At the end of the study, anti-oxidant parameters superoxide dismutase, catalase, reduced glutathione, lipid peroxidation were measured.

5. DISCUSSION AND CONCLUSION

5.1 Effects of *Ethanolic Extract of Alternanthera sessilis* on Final Body Weight

Diabetic rats showed significant decrease in body weight in comparison to normal rats. Diabetic rats treated with ethanolic extract of *Alternanthera sessilis* at doses of 500, 1000 mg/kg bw showed significant improvement in body weight.

5.2 Effects of Ethanolic Extract of *Alternanthera Sessilis* on Final Body Weight, Blood Glucose and Lipid Profile

Diabetic rats exhibited the significant increase in blood glucose, total cholesterol, triglycerides, lipid peroxidation and significant decrease in high density lipoprotein in comparison to normal rats. Diabetic rats treated with ethanolic extract of *Alternanthera Sessilis* at doses of 500 and 1000 mg/kg b w showed significant decrease in blood glucose, total cholesterol, triglycerides, lipid peroxidation and significant increase in high density lipoprotein in comparison to diabetic rats in dose dependent manner.

5.3 Effects of EEAS on Anti-Oxidant Parameters

Diabetic rats exhibited the significant decrease in superoxide dismutase, Catalase, Reduced glutathione in comparison to normal rats. Diabetic rats treated with ethanolic extract of *Alternanthera Sessilis* at doses of 500 and 1000 mg/kg b w showed significant increase in

Superoxide dismutase, Catalase, Reduced glutathione in comparison to diabetic rats in dose dependent manner.

5.4 Effects of Ethanolic Extract of *Alternanthera sessilis* on Lens Aldose Reductase

Diabetic rats exhibited the significant decrease in Aldose reductase in comparison to normal rats. Diabetic rats treated with ethanolic extract of *Alternanthera Sessilis* at doses of 500 and 1000 mg/kg b w showed significant increase in Aldose reductase in comparison to diabetic rats in dose dependent manner [8,9,10].

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ETHICAL APPROVAL

All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethical Committee, Protocol No. IAEC/XV/03/RIPER/2020.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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