

Research Article

Evaluation of Hypoglycaemic Effect and Antioxidant Property of *Entada Abyssinica* Leaves Powder on Alloxan Induced Diabetic Mice

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Abstract: This study examined the hypoglycaemic effect of *Entada abyssinica* leaves on alloxan induced diabetes mellitus. The experiment was conducted using 12 mice for a period of 5 weeks divided into three groups of four mice each. Group 1 was un-induced (positive control), group 2 was induced fed on non-supplemented diet (negative control) and group 3 was induced fed *Entada abyssinica* (tawatsa) leaves supplementation (5% of the diet). The mice were made diabetic by intra-peritoneal injection of 180 mg/kg b.wt with alloxan. Fasting blood glucose was measured seven days after diabetic induction to determine the severity of blood glucose elevation. Similarly fasting blood glucose was measured every week to evaluate the hypoglycaemic effect of the supplement. *E. abyssinica* effects on serum glucose level and malondialdehyde levels were significant in diabetic treated compared to diabetic control (6.7 ± 0.81 vs. 16.2 ± 1.6 mmol/L and 10.08 ± 0.61 vs. 16.44 ± 3.00 μ mol/L at $p<0.05$), respectively. The result of this research suggests that *E. abyssinica* has hypoglycemic and antioxidant effect on alloxan induced diabetic mice.

Keywords: Alloxan, antioxidant, diabetes mellitus, *Entada abyssinica*, hypoglycaemic effect, oxidative stress

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels as a result of defects in insulin secretion, action or both (Fishbein and Palumbo, 1995). Normally blood glucose level is tightly controlled by insulin; a hormone produced by the pancreas. Insulin lowers the blood glucose level. In patients with diabetes mellitus, the absence or insufficient production of insulin causes hyperglycemia (Mathew and Augusta, 2005). The classical symptoms of diabetes are polyuria, polydipsia and polyphagia (Narayan *et al.*, 2003). Oxidative stress has been implicated as the cause of complication in diabetic patients (Ramakrishna and Jaiikhani, 2007; Sharma *et al.*, 2000). Alloxan is used in the induction of chemically induced diabetes mellitus in experimental animals, it exerts its effects via reduction by glutathione to dialuric acid in which redox cycling process generates reactive oxygen species that damage the pancreatic beta-cells (Malaisse, 1982).

Entada abyssinica is a small to medium sized deciduous tree, 3-15 m high, belonging to the family *Fabaceae*, sub family *Mimosoideae*. *E. abyssinica* is used as a traditional medicine to cure many diseases. The plant is used in the treatment of miscarriage and the

leaf for fever, while decoction of the bark is taken for coughs, chronic bronchial enlargement, rheumatic pains and abdominal pain (Bekele-Tesemma *et al.*, 1993). An infusion of crushed roots is used in the treatment of bronchial ailment. A root or leaf decoction is used as a fever remedy, powdered or roasted pulverized seeds for sneezing, root bark as a message for swelling and the raw fruit induces vomiting as an antidote to snake venom (Bein, 1996). The seeds are used to treat cataracts and diseases of the back of the eye and the fresh root is used against Gonorrhoea (Haile and Delenasaw, 2007). *Entada abyssinica* is used in Hausaland for the management of a variety of diseases including Diabetes mellitus. Though, *Entada abyssinica* has been associated with well documented therapeutic benefits (Bein, 1996; Haile and Delenasaw, 2007), there is paucity of information about its potential as anti-hyperglycaemic agent currently employed by Hausa community in the management of Diabetes mellitus. Thus, this study is an attempt to provide evidence on the possible role of the plant in the management of Diabetes mellitus using alloxan induced mice as animal model. The amount of the powdered leaves used was extrapolated from what is given to normal adult per day in Hausaland.

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MATERIALS AND METHODS

Materials:

Plant materials and preparation: Fresh green leaves of *Entada abyssinica* (Tawatsa) were collected from Yamidi college of Agriculture Hadejia Local Government, Jigawa State. And it was identified by Malam Yusuf from Department of The plant leaves were identified by the Botany unit of Biological Sciences Department, Bayero University, Kano, Nigeria The fresh leaves were dried under shade and then grounded in to powder in the laboratory using mortar and pestle. The powder was mixed with the animal feed based on the extrapolation to the amount used in human in the management of Diabetes mellitus.

Experimental animals: The experiment was conducted on 12 mice weighing 22-30 g obtained from Vom Jos, Plateau state, Nigeria. The animals were allowed to acclimatize for 7 days. The animals were kept in cages in an environmentally control animal facility (room temperature, 12 h light, 12 h dark cycle) with free access to a standard commercial diet and water. The weight gain and level of blood glucose were determined on weekly bases. The experiment was conducted for period of 5 weeks

Chemicals and reagents: All the chemicals and the reagents utilized in the research are of analytical grade and products from Sigma (St. Liouis, USA), Randox Laboratories Ltd. Ardmore, United Kingdom.

Methods:

Experimental design and treatment: Twelve mice weighing 22-30 g were used for the experiment. The mice were selected randomly divided in to three groups as follows:

Group 1: (n = 4), control animals non diabetic received normal diet

Group 2: (n = 4), control animals diabetic received normal diet

Group 3: (n = 4), diabetic fed on supplement (5% of *E. abyssinica* leaves)

ALLOXAN preparation and induction: All animals were fed on normal diet for 7 days of acclimatization. Diabetes mellitus was induced by an Intraperitoneal (IP) injection of 180 mg/kg body weight to mice in groups 2 and 3 after 16 h of fasting. Blood glucose levels were measured 48 h after administration by gently nipping the mice tail with a sterilized blade to get one drop of blood which was placed on the sample well of the strip of ACCU Check glucose monitoring meter and confirmed by the use of glucose oxidase methods. Only rats with fasting glucose greater than 11.1 mmol/L were included in the study (Stanley and Venogopal, 2001). The glucometer was calibrated before use using standard glucose oxidase method of Barham and Trinder (1972).

Table 1: Mean weight gain and loss of the experimental mice for 5 weeks

Week	Weight (g)		
	Group 1	Group 2	Group 3
1	26±2.6	29±1.2	27±4.4
2	28±2.7	28±1.0	32±7.6
3	29±2.7	25±1.0	33±6.1
4	31±2.4	23±2.1	34±6.0
5	33±2.2	21±2.1	35±6.1

Result is mean±S.D.; Sample size per group = 3; Group 1: Un-induced; Group 2: Induced-untreated; Group 3: Induced treated

Table 2: Blood glucose profile of mice treated with supplement and control groups on normal diet

Week	Blood glucose level mmol/L		
	Group 1	Group 2	Group 3
1	6.2±1.3	12.7±1.30	15.7±1.20
2	5.9±1.4	13.3±0.81	12.3±0.42
3	5.3±1.1	14.5±1.60	11.6±0.49
4	5.8±1.7	15.2±1.60	9.6±1.80
5	6.4±1.4	16.2±1.60*	6.7±0.81*

Result is mean±S.D.; Sample size per group = 4; p* < 0.05 indicates statistical significance compared with group 2 (induced untreated); Group 1: Un-induced; Group 2: Induced-untreated; Group 3: Induced treated

Table 3: Serum malondialdehyde level in mice on supplemented diet and control

Group	Concentration of serum MDA (nmol/cm ³)
1 non diabetic control	10.61±0.39*
2 diabetic control	16.44±3.00
3 alloxan induced diabetes mellitus on supplementation	10.08±0.61*

Result are mean±S.D.; Sample size n = 2; p* < 0.05 indicates statistical difference compared with group 2 (induced-untreated); Group 1: Un-induced; Group 2: Induced-untreated; Group 3: Induced-treated

Biochemical assay: Fasting blood glucose was estimated by glucose oxidase method according manufacturer's procedure (Randox laboratories Ltd. Ardmore, United Kingdom). Urine glucose was determined using Combostik (DFI Co Ltd, Gimhae, Gyuang-Nam, Korea).

Determination of serum Malondialdehyde (MDA):

Plasma malondialdehyde was measured by the method of Ohakawa *et al.* (1979). Lipid peroxidation generates peroxide intermediates which upon cleavage release MDA, a product which reacts with Thiobarbitutic Acid (TBA). The product of the reaction is colored complex, which absorbs light at 532 nm.

Statistical analysis: Data are expressed as mean±S.D. One-way Analysis of Variance (ANOVA) (SPSS 14.2 software) for continuous variables, to compare data from the three groups analyzed was used. Statistical significance levels of at least p < 0.05 were used for all tests.

RESULTS

Table 1 shows the weekly body weights one week before and 4 weeks after induction of diabetes mellitus. The weekly mean body weights are in grams. Group 1

(Un-induced) and Group 3 (Induced and treated) appeared to have weekly increase in weight but Group 2 (Induced untreated) had reduction in weight at the end of the fifth week.

Table 2 shows result of blood glucose profile for mice treated with *E. abyssinica* and control groups on normal diet for 5 weeks. The results are of mean and standard deviation. There was no significant change between induced group on supplement compared control un-induced group. However, there was a significant increase ($p < 0.05$) in blood glucose level in diabetic untreated control group 16.2 ± 1.6 mmol/L compared with either diabetic induced treated group (6.7 ± 0.81 mmol/L) or non diabetic control group (6.4 ± 1.4 mmol/L).

The Table 3 shows the result of serum MDA at the end of 5 week experiment. The results were represented as Mean \pm S.D. of serum MDA. It indicates statistical significance difference between group 2 (induced-untreated) (16.44 ± 3.00 nmol/cm³) and group 3 (induced treated) (10.08 ± 0.61 nmol/cm³) at $p^* < 0.05$. Significant reduction of MDA level in group 3 may indicate that *E. abyssinica* possesses antioxidant property.

DISCUSSION

Several lines of evidence suggest that diabetes mellitus and complication thereof from the disorder can arise due to oxidative stress. Similarly increased lipid peroxidation has been seen in many animal models of diabetes mellitus (Anjaneyulu and Chopra, 2004). However, since the understanding that the selective destruction by alloxan of pancreatic beta cells is mediated via generation of oxidative stress (Dunn and McLetchie, 1943), interest has been stimulated in the antioxidants to evaluate their potentials to prevent alloxan induced diabetes mellitus. *E. abyssinica* is being used in Hausaland as hypoglycemic agent and has been known to be rich in antioxidants such as flavanoids (Asfaw *et al.*, 2000). In this study *E. abyssinica* reduced blood glucose level in alloxan induced diabetic mice. Hence this research study provided insight as to the potential of *E. abyssinica* dietary supplementation in the treatment of chemically induced diabetes mellitus in mice. Similarly the antioxidant property of the plant was demonstrated by its ability to reduce blood MDA concentration as compared with diabetic non-treated and supplemented mice group.

Previous studies have shown that biologically active substances obtained from *E. abyssinica* including diastereoisomer of clerodane type diterpene, kolavenol (Freiburghans, *et al.*, 1998), flavanoids, tannins and phytosterol glycosides (Asfaw *et al.*, 2000) and kolavinc acid derivatives (Nyasse *et al.*, 2004). There is reservoir of basic information that establishes the hypoglycaemic effect of flavanoids. Hence the glucose

lowering effect of the plant may be associated with the presence of flavanoids. Diabetes mellitus is a disorder associated with oxidative stress, in this study; the plant has the ability to reduce serum MDA level, a potential indicator of oxidative stress. Hence its therapeutic advantage in the management of diabetes mellitus may be associated with its ability to attenuate oxidative damage of tissues in diabetic subject.

CONCLUSION

In conclusion, the results provide the first physiological evidence that *E. abyssinica* possesses anti-hyperglycemic effect in alloxan induced diabetic mice that may offer a valuable therapeutic measure in the treatment of diabetes mellitus. The result of this study is suggestive that *E. abyssinica* may be used as an adjunct regime in treatment of diabetes mellitus as it shows the potential of reducing blood glucose.

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