Evaluation of Anti-diabetic Potential of Ethanol Leaf Extract of *Jathropha Tanjorensis* and Impact on Liver Antioxidant Enzymes

Abstract

The aim of this study was to evaluate the anti-diabetic property of ethanol leaf extract of *J. tanjorensis* and effect on liver antioxidant enzymes. Freshly harvested leaves of *J. tanjorensis* were thoroughly washed with tap water to get rid of debris and afterwards dried at room temperature before being ground into fine powder prior to extraction. Twenty five (25) adult male wistar rats were divided into five groups of five rats per group. **Group I** was the normal control and was allowed unrestricted access to food and water only. **Group II** was the negative control and was induced with diabetes without treatment, **Groups III and IV** were diabetic rats treated with ethanol leaf extract of *J. tanjorensis*, while **Group V** was diabetic rat treated with the standard drug. After treatment had been concluded, animals were sacrificed and blood and tissue harvested were analysed using standard procedure. A significantly ($P<0.05$) high blood sugar level was reported for diabetic rats which however was significantly ($P<0.05$) reversed with oral administration of ethanol leaf extract of *J. tanjorensis* in a dose dependent manner. The activity of the liver antioxidant enzymes was significantly ($P<0.05$) reduced in diabetic rats which however was significantly ($P<0.05$) increased following oral administration of *J. tanjorensis* leaf extract in a dose dependent manner across treatment periods. In conclusion, the study unveils the antidiabetic potential of *J. tanjorensis* leaf.

Keywords: ***Jathropha tanjorensis,*** antioxidant enzymes, Anti-diabetic, Diabetes, Liver

Introduction

Diabetes mellitus (DM) is a disease condition which is characterized by an abnormally level of sugar in the blood. Diabetes mellitus abound globally and has been identified as the one of most lethal disease of mankind. An estimated 4.4% of the world population is projected to suffer from diabetes in 2030 [1].

Diabetic neuropathy, nephropathy and retinopathy among others have been implicated in prolonged diabetes mellitus [2] while complications arising from the condition are hypertention, artherosclerosis and micro circulatory disorders [3].

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In diabetic patients, mononuclear cells are effectively engaged in the generation of reactive oxygen species (ROS) and consequently, macromolecules such as lipid, protein and DNA are damaged [4].

Jathropha tanjorensis is a member of the *Euphorbiaceae* family. It is cultivated widely in the Southern part of Nigeria. Interest among researchers in the aforementioned plant has grown over the years owing to its immense health significance, availability and affordability [5]. Analysis on the leaf of of *Jathropha tanjorensis* unveiled the presence of alkaloids, flavonoids, tannins, cardiac glycoside, anthroquinones and saponins [6]. Reports abound on the antihypertensive, antioxidant, antimicrobial, antimalarial and hypolipidemic activity of *Jathropha tanjorensis* [7]. Hence, the need to probe the leaf further in an effort to unveil more health benefits of the said plant.

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Materials and Methods

Collection of plant material

Fresh leaf of *Jathropha tanjorensis* freshly obtained a local market was identified at the herbarium unit of the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State.

Sample Preparation

Leaves of *J. tanjorensis* were air dried at room temperature for three days after which they were ground to fine powder with the aid of an electric blender. The powdered sample was stored in a

moisture free, air-tight container until further use. 500 g of powdered plant sample was soaked in 96% ethanol for 2 hr. The extract obtained was filtered and concentrated with a rotary evaporator. The brownish residue obtained was dried in desiccators

Animals

Adult male wistar rats weighing between 150-250 g were obtained from the animal house of Abia State University Uturu. The rats were housed and maintained in well ventilated plastic cages under standard laboratory conditions and were allowed unrestricted access to food and water. Acclimatization of the animals occurred within three weeks before the experiment.

Median Lethal dose 50% (LD50%)

The LD 50% was determined using three groups of three wistar rats and was each subsequently administered with 10, 100 and 1000 mg/kg of extract orally. Animals were studied for 24 hr to observe signs of toxicity. Upon confirmation of absence of mortality in any of the groups, another three groups of one rat each was each administered with 1600, 2900 and 5000 mg/kg of extract separately. The animals were observed for 48 hr for signs of toxicity Lorke [8].

Animal Grouping

A total of 25 rats divided into 5 groups of 5 rats each were used in this study.

Group 1: Non diabetic without treatment. (Normal control)

Group 2: Diabetic rats without treatment (Negative control)

Group 3: Diabetic rats treated with 200 mg/kg .bw *J. tanjorensis leaf* extract.

Group 4: Diabetic rats treated with 400 mg/kg .bw *J. tanjorensis* seed extract.

Group 5: Diabetic rats treated with 2.5 mg/kg Metformin daily.

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Induction of Diabetes

Induction of diabetes mellitus was by a single intraperitoneal injection of 120 mg/kg body weight of alloxan [9] and blood sugar level evaluated with the aid of a glucometer (Acc-cheek Advantage Roche diagnostics GmbH, Germany) after three days and the rats with fasting blood glucose level in excess of 126 mg/dl (11.1mmol/L) were deemed elegible for the study.

Preparation of Liver Homogenate

In 1:5 of0.9% sodium chloride (ice cold), the liver tissue was homogenize and subsequently centrifuged at 3500 rpm for 20 minutes to obtain the supernatant which was sed to assay for the activity of superoxide dismutase (SOD) and catalase (CAT).

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Biochemical analysis

Determination of Superoxide Dismutase Activity

The method described by Martin et al [10] was employed to determine the superoxide dismutase activity. Exactly 920 µL of phosphate buffer (0.05 M, pH 7.8) was added to 40 µL of the sample. A reagent test was prepared by replacing the sample with 40 µL of sample dilution buffer (0.85% NaCl). The mixtures were incubated for 2 min at 25^0C before 40 µL of hematoxylin was added. Following the addition of 40 µL of hematoxylin, absorbance of the sample test and reagent test was read at 560 nm immediately and after 5 minutes against the sample blank which is distilled water.

SOD concentration in the sample was calculated thus;

Absorbance Reagent test (AR) = Absorbance Reagent test 2 − Absorbance Reagent test 1

Absorbance sample test (As) = Absorbance sample test 2 − Absorbance sample test 1

% inhibition = [1− As / AR] x 100

SOD (µ/ml) = [1− As / AR] x 100 x 1.2

Determination of Superoxide Dismutase Activity

To 1 ml of reaction mixture containing 1.96 mL phosphate buffer (0.01 M, pH 7.0), 1.0 mL hydrogen peroxide (0.2 M) and 0.04 mL of homogenate in a final volume of 3.0 mL. 2 ml of dichromate acetic acid reagent was introduced and subsequently heated for 10 minutes before being cooled and absorbance read at 570 nm [10].

Statistical Analysis

Data were analysed using one way Analysis of Variance (ANOVA). Differences in mean were compared using Turkey Test. *p-values* less than 0.05 was considered statistically significant.

Table 1: Blood Glucose Levels of Diabetic rats treated with Ethanol Leaf extract of *J. tanjorensis*

Groups	Treatment	Blood Glucose Levels (mg/dl)		
		Week 1	**Week 7**	**Week 14**
Group I	Normal control	98.12±2.05^{a}	97.89±3.70^{a}	98.02±3.05^{a}
Group II	Diabetic-treatment	230.20±3.70^{a}	229.10±3.20^{a}	231.05±0.05^{a}
Group III	Diabetic+200 mg/kg extract	184.20±3.60^{c}	136.21±3.02^{b}	120.81±3.41^{a}
Group IV	Diabetic+400 mg/kg extract	110.00±4.61^{c}	106.21±2.02^{b}	100.00±32.02^{a}
Group IV	Diabetic+Std mg/kg extract	102.10±2.22^{c}	100.31±20.13^{b}	98.87±4.11^{a}

Results are expressed as mean ± Standard deviation of three determinations. Values with different superscript in a row are significantly (P<0.05) different.

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Table 2: Activity of Liver Antioxidant Enzymes in Diabetic rats treated with Ethanol Leaf extract of *J. tanjorensis*

GROUPS	TREATMENT	Enzyme activity	
		SOD (U/ml)	CAT(U/ml)
Group I	Normal control	2.60 ± 2.02^{c}	49.33 ± 2.40^{d}
Group II	Diabetic-treatment	0.90 ± 3.20^{a}	32.00 ± 0.58^{a}
Group III	Diabetic+200 mg/kg extract	1.60 ± 1.20^{b}	41.00 ± 1.15^{b}
Group IV	Diabetic+400 mg/kg extract	1.76 ± 1.23^{bc}	41.33 ± 2.60^{b}
Group IV	Diabetic+Std mg/kg extract	2.00 ± 1.30^{c}	45.66 ± 2.73^{c}

Results are expressed as mean ± Standard deviation of three determinations. Values with different superscript in a column are significantly (P<0.05) different.

RESULTS AND DISCUSSION

Diabetes mellitus is a multifactorial disease in which blood sugar level is exceeds the normal range [11]. It is characterized by a deficiency in antioxidant enzymes [12]. Table 1 shows the blood glucose levels of diabetic rats treated with ethanol leaf extract of *Jathropha tanjorensis*. An increased blood sugar level which progressed with time was reported in diabetic rats. However, oral administration of ethanol leaf extract of *J. tanjorensis* significantly (P<0.05) caused a reduction in the blood sugar level of diabetic rats in a dose dependent manner. The antidiabetic potential of the aforementioned plant could be attributed to the presence of polyphenolic compounds which had been reportedly present in in leaf of the aforementioned plant [13]. This result is consistent with the finding of Asuk et al. [14] which established that the ethanol-methanol extracts of the leaf, stem, bark and root of *Jatropha curcas* demonstrates appreciable anti-diabetic property. There is a correlation between diabetes and oxidative tissue damage arising from free radical generation [15]. Diabetes causes increased free radical generation which results in the alterations of the liver tissue superoxide dismutase (SOD) and catalase [15]. Table 2 shows the activity of liver antioxidant enzymes in diabetic rats treated with leaf extract of *Jathropha tanjorensis* showing a significantly (P<0.05) decreased activity of liver antioxidant enzymes in diabetic rats which however was significantly (P<0.05) increased following oral administration of *J. tanjorensis* leaf extract in a dose dependent manner across treatment periods. This could be attributed to the antioxidant property of the plant extract which creates a balance between free radical generated and eliminated. This result is in tanden with the

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finding of Asuk et al [14] which reported the antioxidant activity of the leaf, root and stem of *Jatropha curcas* a member of the *Euphorbiaceae* family to which *Jatropha tanjorensis* belongs.

Conclusion

It is evident that the leaf of *Jatropha tanjorensis* wields impressive antidiabetic and antioxidant properties and thus should be analysed further to unveil the active components characteristic of the said function for drug development purposes.

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