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Research Article
Hypoglycaemic Efficacies of Leaf and Stem Extracts of *Jatropha tanjorensis* (Euphorbiaceae) in Diabetic Mice

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Abstract

Background and Objective: In Nigeria, *Jatropha tanjorensis* is used for preparing local herbal medicine for managing diabetic conditions. However, few quantitative data exist to authenticate these widely acclaimed efficacies. This study was carried out to compare the anti-diabetic activities of aqueous and ethanolic extracts of leaf and stem of the plant. **Material and Methods:** Phytochemical analyses of the plant parts were investigated following standard protocols. Seventy mice of both sexes were randomly divided into 7 groups of 10 mice: Group 1 (normal control) administered normal saline, group 2 (diabetic/negative control) with no treatment, group 3 (positive control) treated with standard drug, while groups 4-7 received 200 and 400 mg kg^{-1} b.wt., of the extracts. Treatments was daily via oral intubation for 10 days, following standard protocols. Blood glucose levels and body weights were determined on days 1, 5 and 10 post-treatment. **Results:** Phytochemical analyses revealed various bioactive components based on the plant part and solvent of extraction. The results revealed successful induction of diabetic condition in the mice by Alloxan. All plant extracts showed significant hypoglycaemic activities irrespective of solvent of extraction and plant parts. Aqueous stem extracts induced the highest reduction in glucose level at day 10. In all treatments, initial weight loss on day 5 was followed by gain on day 10 post-treatment. **Conclusion:** This study, thus, revealed anti-diabetic potentials of the plant parts and supports earlier inclusion and use of the plant against pathogenic micro-organisms and management of diabetic conditions.

Key words: Hyperglycaemic, hypoglycaemic, alloxan, *Jatropha tanjorensis*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM), commonly called diabetes, is a degenerative metabolic disease condition, characterized by high sugar levels over a prolonged period[1]. Initially perceived as a disease of the aged, the disease is gradually becoming a common phenomenon among relatively young people[2]. Diabetes Mellitus is commonly associated with frequent urination, increased thirst and hunger. When left untreated, serious complications such as diabetic ketoacidosis, non-ketotic hyperosmolar coma can occur[3]. Other long-term complications of the disease include cardiovascular disease, stroke, chronic kidney failure, foot ulcer and damage to the eye[4]. In 2006, an estimated 387 million people have diabetes worldwide, with an estimated death of 1.5-4.9 million yearly[5].

The treatment of diabetes, generally, involves the development of drugs that aims, basically, at reducing the high glucose level to a physiological level for proper functioning of body cells[6]. However, there are records of patients who either use these drugs or complement them with local or traditional therapies. The use of complementary ethno-botanical therapies for treatment of diabetes is as old as man himself and is still widely used today[7]. The incessant cases of drug complications and resistance informed call for development of drug products, especially of plant origin. This is pivoted on the long history of their use in curing human ailments.

More so, drug products of plant origin are cost-effective, available, accessible and effective[2]. Although, traditional plant remedies or herbal formulations exist from ancient times and still widely used, a lot of controversies have trailed their efficacies, claims and safety[7]. There have also been concerns raised about their bio-safety and efficacy[8]. These concerns have necessitated scientific studies to ascertain the widely acclaimed hyper or hypoglycaemic efficacies of extracts of these ethno-botanicals.

In the search of plant formulation for treating hypoglycaemic and hyperglycaemic conditions all over the world, several ethnobotanicals have been screened, with some of their secondary metabolites (e.g., alkaloids, glycosides, terpenes, flavonoids) isolated. In Nigeria, for example, among the many ethnobotanicals used in the treatment of diabetes and related conditions is *Jatropha tanjorensis*. This plant, commonly called 'hospital-too-far', catholic vegetable, 'Iyana-Ipaja' or 'Lapalapa', belong to the family Euphorbiaceae. It is a common weed of field crops, bush regrowth and road sides[9]. Locally, it is a source of edible leafy vegetable and as medicinal plant[10], its extracts, especially, having hypoglycaemic and antioxidant properties[11]. Extracts of the plant improve haematological indices, possess anti-plasmodial properties[12] and larvicidal potency[13].

Despite these feats, adequate laboratory testing has been emphasised and advocated to substantiate these claims[14]. This present study, therefore, aims at, quantitatively, determining the efficacy of the plant in managing high glucose level conditions. More so, to compare the anti-diabetic efficacies of different extracts of plant parts (stem and leaves) used locally for treating diabetic conditions. Hence, a panacea for the development of lead drug agent for the management of DM.

MATERIALS AND METHODS

Collection, authentication, preparation and extraction of plant materials: Fresh leaves and stem-cuts of *Jatropha tanjorensis* were collected from residential areas in Minna, Nigeria (longitude 6°33 E and latitude 9°27 N). The plant was identified by a botanist of the Department of Plant Biology, Federal University of Technology, Minna (where voucher specimens of the leaves were deposited (VN: JT/83/16)). The plant parts were dried under shade in the laboratory for 14 days and pulverised using an electric blender (Model no: QASA QLB-20L40).

To obtain ethanolic extracts of the leaf and stem, the pulverised materials were introduced into extraction chamber of Soxhlet extractor for 48 h at 70°C using ethanol as solvent. For aqueous extracts, the pulverised materials were soaked in distilled water for 72 h and agitated until a deep colour of the extract was obtained. The extracts produced were concentrated to dryness on water bath and then weighed[15].

Phytochemical screening of the crude extracts of *J. tanjorensis*: Qualitative phytochemical screening of the crude extracts of the leaf and stem were carried out on the ethanolic (JELE) and aqueous (JALE) leaf extracts of the plant using standard procedures[15] (Plate 1, 2).

Plate 1: Fresh Stem and leaves of *Jatropha tanjorensis*
(Source: Field photography)

Plate 2: Dried and pulverised (a) Stem and (b) Leaf of *J. tanjorensis*
(Source: Field Photography)

Anti-diabetic investigation of the extracts of *Jatropha tanjorensis*

Animal husbandry: Seventy mice of both sexes weighing between 20-38 g were used in the study. Treatments with the plant extracts was done daily via oral intubation for 10 days, following standard protocols. The mice were fed with standard mice feed and administered water *ad libitum*. However, the animals were starved on the night prior to the commencement of experimentation. All animal experiments were conducted in compliance to standardized protocols approved by the Ethics Committee of General Hospital, Minna, Niger state.

Induction of diabetic condition in the experimental animals: Diabetic conditions were induced in the animals (60 mice) by a single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (120 mg kg^{-1} b.wt.,). Three days later, mice with blood glucose concentration above 120 mg dL^{-1} were considered diabetic[16].

Determination of blood glucose levels and body weight: Ten non-diabetic mice served as the normal control (group 1) and administered normal saline equivalent to the doses of the extracts. The diabetic mice (60) were divided randomly into 6 groups of 10 mice each. Mice in group 2 served as the diabetic control and received no treatment, while those in group 3 (positive control) were treated with standard drug (Metformin, 250 mg kg^{-1}). Groups 4 and 5 received, respectively, 200 and 400 mg kg^{-1} b.wt., of the ethanolic and aqueous extracts of the stem, while groups 6 and 7 received 200 and 400 mg kg^{-1} b.wt., of the ethanolic and aqueous extracts of the leaf. All treatments were done daily via oral route and lasted for 10 days, with body weights taken on days[16] 1, 5 and 10.

Table 1: Phytochemical components of crude extracts of *Jatropha tanjorensis*

	Stem		Leaf*	
Components	Aqueous	Ethanolic	Aqueous	Ethanolic
Cardiac glycosides	++	+	-	-
Alkaloids	+	-	++	+
Saponins	-	++	-	+
Steroids	+	-	+	-
Tannins	-	-	+	+
Phenols	-	-	+	+
Flavonoids	+	+	-	-
Terpenes	+	-	-	-
Anthraquinones	++	-	-	+
Emodols	+	-	-	-

Not detected, +: Fairly present, ++: Moderately present, *Source: Daniyan *et al.*[15]

Data analysis: Results obtained from the study were expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA), students t-test at 95% level of significance were used to determine significant differences between controls and treated group. Duncan Multiple Range Test (DMRT) was used to separate these differences.

RESULTS

Phytochemical screening of the crude extracts of leaf and stem of *Jatropha tanjorensis*: Phytochemical analyses of aqueous and ethanolic stem and leaf extracts of *J. tanjorensis* are shown in Table 1. It revealed the presence of bioactive components. These components include cardiac glycosides, alkaloids, saponins, steroids, tannins, phenols, flavonoids, terpenes, anthraquinones and emodols. While alkaloids were found in all extracts except ethanolic stem extract, emodols and terpenes were found only in aqueous stem extracts of the plant. Also, while flavonoids and cardiac glycosides were found only in the extracts (aqueous and ethanolic) of the plant's stem, Tannins and phenols were present only in the leaf extracts of the plant. More so, only aqueous extracts of both plant parts contained steroids (Table 1).

Effects of crude extracts of *J. tanjorensis* on blood glucose level of alloxan-induced diabetic mice: The effects of administration of crude extracts (aqueous and ethanolic) of stem and leaf of *J. tanjorensis* on the blood glucose levels of Alloxan-induced diabetic mice are shown in Fig. 1. The results revealed a successful induction of hyperglycaemic conditions in the mice using Alloxan monohydrate. Analysis showed that on day 5 post-treatment, there were significant ($p<0.05$) reduction in the blood glucose level of all treated mice. This plummeted further on day 10 post-treatment (Fig. 1).

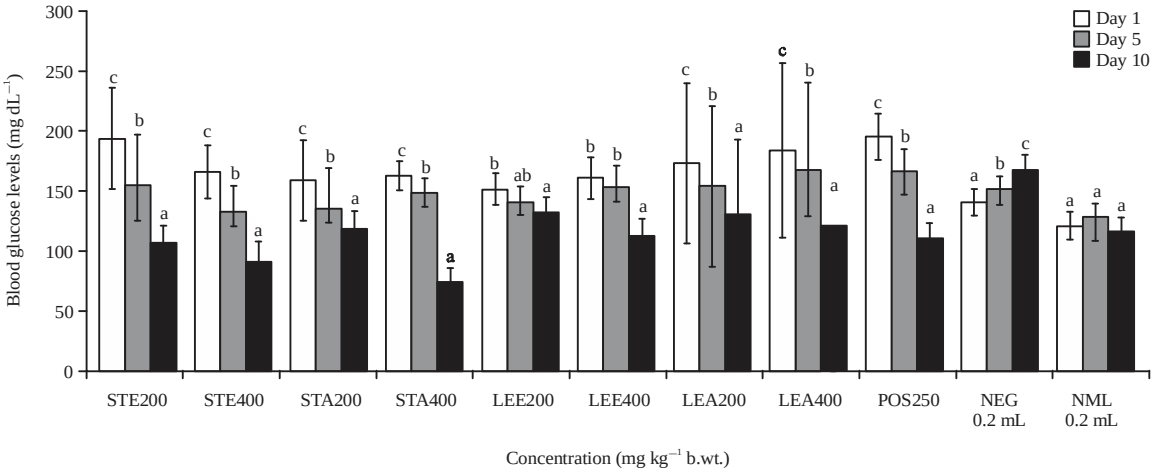
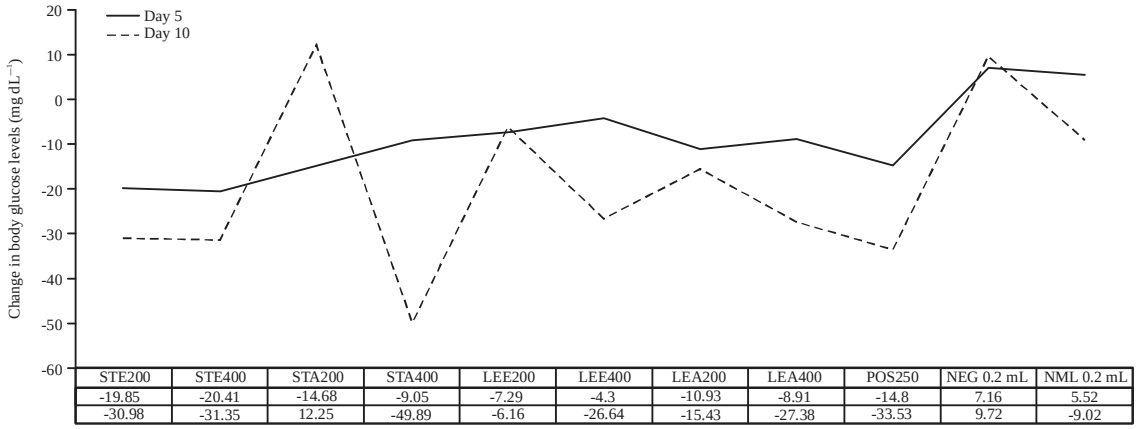


Fig. 1: Blood glucose levels of mice treated with aqueous and ethanolic steam and leaf extracts of *Jatropha tanjorensis* at days 1, 5 and 10. Bars with same letter for an extract type are not significantly different at $p<0.05$ according to analysis of variance

STE: Ethanolic stem extract, STA: Aqueous stem extract, LEE: Ethanolic leaf extract, LEA: Aqueous leaf extract, POS: Positive control, NEG: Negative control, NML: Normal control, 0.2, 200, 250 and 400 = Concentrations (mg kg^{-1} b.wt.), Values are expressed as Mean±SD



STE200	STE400	STA200	STA400	LEE200	LEE400	LEA200	LEA400	POS250	NEG 0.2 mL	NML 0.2 mL
-19.85	-20.41	-14.68	-9.05	-7.29	-4.3	-10.93	-8.91	-14.8	7.16	5.52
-30.98	-31.35	12.25	-49.89	-6.16	-26.64	-15.43	-27.38	-33.53	9.72	-9.02

Fig. 2: Change in body glucose level at days 5 and 10 post-treatment with aqueous and ethanolic extracts of steam and leaf of *Jatropha tanjorensis*

STE: Ethanolic stem extract, STA: Aqueous stem extract, LEE: Ethanolic leaf extract, LEA: Aqueous leaf extract, POS: Positive control, NEG: Negative control, NML: Normal control, 0.2, 200, 250 and 400 = Concentrations (mg kg^{-1} b.wt.)

However, mice cohorts treated with aqueous stem extract (400 mg kg^{-1} b.wt.,) had the lowest glucose level at the final day of treatment (day 10) (Fig. 1).

On day 5 of treatment with the plant extracts, there was no much variation in change in glucose levels, except with leaf ethanolic extract at 400 mg kg^{-1} b.wt.,. Although, in the negative control, this was slightly higher. On day 10, however, significant changes in glucose levels were recorded with the extracts of *J. tanjorensis* with aqueous stem extract (200 mg kg^{-1} b.wt.,) eliciting the highest change (Fig. 2).

Effects of crude extracts of *J. tanjorensis* on body weight of alloxan-induced diabetic mice: The effect of administration of crude aqueous extracts of *J. tanjorensis* on the body weight of Alloxan-induced diabetic mice is shown in Fig. 3. The result revealed a significant ($p<0.05$) initial decrease in body weight on day 5, which increased significantly on day 10 of treatment for all extracts tested.

DISCUSSION

Phytochemical screening of the crude extracts of the leaf and stem of *J. tanjorensis* revealed a total of 10 bio-active

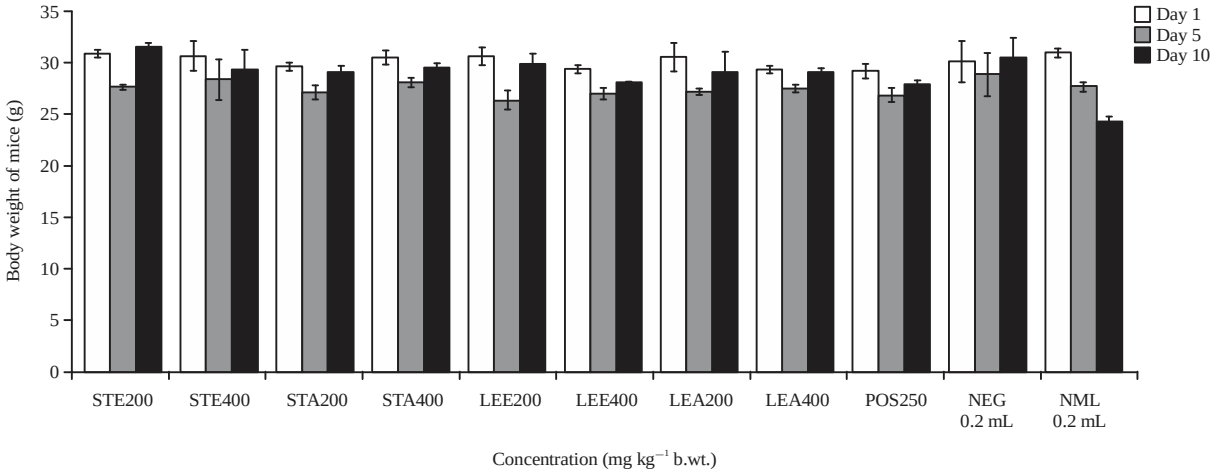


Fig. 3: Body weight changes due to administration of aqueous and ethanolic steam and leaf extracts of *Jatropha tanjorensis* in Alloxan-induced diabetic mice

STE: Ethanolic stem extract, STA: Aqueous stem extract, LEE: Ethanolic leaf extract, LEA: Aqueous leaf extract, POS: Positive control, NEG: Negative control, NML: Normal control, 0.2, 200, 250 and 400 = Concentrations (mg kg^{-1} b.wt.), Values are expressed as Mean±SD

components. However, the presence of these compounds varied with plant part and solvent of extraction. For example, the stem revealed a total of 8 different components, 7 of which were found in the aqueous extract and 3 only in the ethanolic extracts. Likewise, the leaf revealed six components, with three in both extracts and two exclusively in the ethanolic extract.

The presence of these bio-active components could be responsible for activities of the plants. For example, against micro-organisms[17,18] as an antimalarial, analgesic, stimulants and anti-sickling agents[19]. More so, saponins present in the plant have been found to be beneficial in managing hypercholesterolemia[20], while, cardiac glycosides are potent in curing cardiac insufficiency, coughs and circulatory problems and as good sedative with having anti-spasmodic properties[21]. Tannins, on the other hand are used to combat diarrhoea, headache, poor appetite, haemorrhoids, heal wounds as bactericides[22-24]. The presence of phenol makes the plant an excellent oral analgesic/anaesthetic[25]. Flavonoids components may confer on the plant an anti-allergic, anti-inflammatory, anti-oxidant[26,27], anti-microbial, anti-bacterial[28], anti-fungal, anti-viral, anti-cancer, anti-diarrheal activity and anti-anaemic potential[29,30].

In the present study, Alloxan monohydrate successfully induced hyperglycaemia in the mice used, similar observation had been made earlier by Ijioma *et al.*[2]. This hyperglycaemic condition is the foremost sign in the development of diabetes mellitus and generally leads to the lowering of insulin production and secretion, with its attendant lowering of the rate of conversion of glucose to glycogen[31]. According to Ijioma *et al.*[2], this feat was achieved by the selective destruction of pancreatic beta cells of islets of Langerhans in the animal and concomitant marked increase of sugar levels in the diabetic mice.

All doses of the extracts (aqueous and methanolic) of the parts (leaf and stem) of *Jatropha tanjorensis* plant significantly lowered blood glucose level on day 5 of treatment, tilting towards the normal values at day 10 of the treatment. The results, therefore, suggest that the plant has an anti-diabetic effect on the mice, also, irrespective of the part of the plant or solvent of extraction, this effect was the same. Interestingly, it is worth noting, that this tendency of restoring the glucose level is different with respect to the part of plant and solvent of extraction.

The hypoglycaemic attribute of the plant as observed in the present study may have been achieved by inducing increased insulin secretion, peripheral utilization of glucose in the mice, inhibition of endogenous production, inhibition of intestinal glucose absorption and/or regeneration of existing beta cells[2,31].

The effect of administration of the plant extracts on the body weight showed a general reduction in weight on day 5 of treatment, this was, however, increased on day 10 of treatment. This initial reduction in weight could be as a result of the released glycogen store and/or reduction in glycogen storage associated with hyperglycaemic conditions, however, the general increase observed on day 10 of treatment could be attributed to the hypoglycaemic activities of the plant extract/increased glycogen storage.

CONCLUSION

The study indicates that aqueous and ethanolic leaf and stem extracts of *Jatropha tanjorensis* has hypoglycaemic properties and could be a very safe and potent agent to be employed in the fight against diabetes mellitus.

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