Original Article

Combinatorial Effects of Aqueous Root Extract of *Jatropha Curcas* and *J. Gossypiifolia* in Alloxan-Induced Diabetic Rats

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Received: 23.06.2016 *Accepted: 27.07.2016*

ABSTRACT

Background: Combinatorial effects of aqueous root extract of *Jatropha curcas* (Jc) and *J. gossypiifolia* (Jg) in alloxan-induced diabetic rats was investigated in this research.

Methods: Thirty-six wistar rats were randomized into six groups of six animals each. Group I (control) was not induced but received 0.5 ml of distilled water. Groups II, III, IV, V and VI were induced with diabetes mellitus using alloxan monohydrate and received 14.2 mg/kg body weight (b/wt) glucophage, 0.5ml of distilled water, 250 mg/kg body weight of the root extracts of *J. curcas*, *J. gossypiifolia* and the combined extract respectively for 15 d.

Results: The mixture of *J. curcas* and *J. gossypiifolia* in alloxan-induced diabetic rats resulted in significant reduction in the blood glucose between 39.7% reduction by day 3 and 73.3% reduction by day 13 ($P<0.05$). The abnormal levels of serum and liver enzymes in the diabetic group reflected the significant alteration of liver function by alloxan monohydrate and administration of the mixture was found to restore each enzyme activity to a level that compared well with glucophage. The serum lipid levels were also restored to near normal by this mixture for all the evaluated parameters.

Conclusion: The mixture of roots of *J. curcas* and *J. gossypiifolia* has a greater potential for effective antidiabetic activity compare with individual plant extracts and may be safe for consumption.

Keywords: Alloxan, Diabetes, *Jatropha Gossypiifolia, Jatropha Curcas*, Mixture.

IJT 2017 (1): 11-17

INTRODUCTION

Many patients and health care practitioners have shown great interest in herbal products since about 70% of population worldwide relies on herbal medicines for part of their primary health care [1]. Herbal products are used as single herb, combination of herbs, or combination of herb(s) and drug(s) in different regions and cultures. The effects of herbs in combination can be complicated as various interactions can occur among the individual components. An extensive growth in the field of herbal mixtures is presently gaining ground, and these mixtures are gaining popularity both in developing and developed countries because of their natural origin and lesser side effects [2]. Additional therapeutic benefit is the most desirable result from herbal interactions. This is often the intended or expected outcome when using combination therapy. However, the effects arising from herb-herb or herb-drug interactions are often unpredictable and complicated due to the presence of multiple components in the herbal products [3]. Pharmacologically, interactions among herbs in a multi-item prescription can occur either pharmacokinetically or pharmacodynamically.

Lack of scientific and clinical data proving their efficacy and safety has been the major hindrance in amalgamation of herbal medicine in modern medical practices. Conducting clinical research in herbal mixtures, development of simple bioassays for biological standardization, pharmacological and toxicological evaluation and development of various animal models for toxicity and safety evaluation is highly required. Establishing the active components of these herbal extracts is also important [4].

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Diabetes mellitus is a chronic metabolic disease with life-threatening complications. The International Diabetes Federation (IDF) estimates that 285 million people, i.e., 6.4% of the world population, suffered from diabetes in 2010 and this prevalence will increase to 439 million people (7.7%) of the world population by 2030 [5]. Over 90% of diabetic patients are diagnosed with type 2 diabetes (T2D) [6, 7]. The cost of health care associated with diabetes continues to grow and is a huge economic burden for afflicted patients and countries.

Jatropha curcas is commonly known as physic nut. Aqueous root extract of *J. curcas* exerted hypoglycaemic action and ameliorate anaemic condition in alloxan-induced diabetic rats [8]. *J. gossypiifolia*, commonly known as bellyache bush, used in traditional medicine throughout its native and introduced habitat for the treatment of ailments ranging from fever to diabetes and cancer [9]. A mixture of *J. curcas* and *J. gossypiifolia* is traditionally used for the treatment of diabetes. However, no scientific evidence has been recorded to proof the efficacy of the mixture even though individual plants have been scientifically tested to be potential antidiabetic agents.

In this experimental study, the aim was to examine the antidiabetic activity of herbal mixture comprising of *J. curcas* and *J. gossypiifolia* in alloxan- induced diabetic rats. The potential toxic effect of these plants in the diseased state was also investigated. The interest in studying herbal products is further supported by the observations that many herbal extracts show superior (beneficial synergistic) effect when compared to single chemical constituents at the equivalent dose (or concentration).

MATERIALS AND METHODS

Plant Materials

Fresh plant materials (roots of *J. curcas and J. gossypiifolia*), were collected from a garden at Elega's Compound, Oke-Oyi, Ilorin-East Local Government of Area of Kwara State, Nigeria. The plants were authenticated by Mr Bolu Ajayi, in the Herbarium, Plant Biology Department, University of Ilorin, Ilorin. The voucher numbers: UIH/978/1030 and UIH/719/1031 were given respectively.

Experimental Animals

Thirty-six Wistar rats of both sexes (135±25 g weight) were obtained from the small Animal Breeding Unit, Department of Biochemistry, University of Ilorin, Ilorin. They were maintained on standard animal pellets and water ad libitum.

Preparation of Extracts

Based on simulation from the method of preparation of the extract locally, root of each of the plants (singularly) and mixture of the roots of the plants (ratio 1:1) were subjected to boiling to obtain their extracts. After cooling, the extracts were filtered and the resulting filtrates were concentrated using a Rotary Evaporator. The concentrates were then reconstituted using distilled water.

Induction of Diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared alloxan (150 mg/kg body weight) in ice cold 0.9% NaCl solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Control (normal) rats were not injected with alloxan and were placed on normal saline alone. After 24 h, rats with moderate diabetes having hyperglycaemia (blood glucose level between 200 and 400 mg/dL) were selected and considered as diabetic and used for the experiment. The reconstituted aqueous extracts were administered orally at concentrations of 250 mg/kg body weight rats/ day for 15 d.

The experiment was approved by Research Ethics Committee of the Faculty of Life Science University of Ilorin, Nigeria.

Experimental Groupings

The animals were divided into 6 groups of 6 animals each for the evaluation of anti-diabetic activity.

Group I: Non-diabetic rats administered normal saline for 15 days (Normal-NDDW).

Group II: Diabetic control rats administered normal saline for 15 days (DDW).

Group III: Diabetic rats given Glucophage (14.2 mg/kg/rat/d) in aqueous solution orally for 15 days (DSD).

Group IV: Diabetic rats administered aqueous extract of *J. curcas* roots (250 mg/kg/rat/day) orally for 15 days (DJc250)

Group V: Diabetic rats administered aqueous extract of *J. gossypiifolia* roots (250 mg/kg/rat/d) orally for 15 days (DJg250)

Group VI: Diabetic rats administered aqueous extract of mixture of *J. curcas* and *J. gossypiifolia* roots (250 mg/kg/rat/d) orally for 15 days (DM250)

All tested groups were compared with the group of untreated diabetic rats (DDW).

Determination of Blood Glucose Level

Fasting blood glucose levels were determined with ONE TOUCH BASIC® Glucometer (LIFESCAN, Inc 2001 Milpitas, CA 95035, USA). The fasting Blood Glucose Levels (BGL) was monitored at two days interval for 15 d by tail tipping method. This result was used to calculate the percentage reduction in blood glucose level using the formula:

% Reduction in Blood Glucose Level (BGL)

$$= \frac{BGL_1 - BGL_n}{BGL_1} \times 100$$

Where n = Day 1, 3, 5, 7, 9, 11, 13 and 15

Biochemical Studies

After 15 d of extract administration, the rats were humanely sacrificed by anaesthetization and the neck area was quickly cleared of fur before the jugular vein was sharply cut with sterile surgical blade. Blood samples (5 ml from each rat) were drawn by cardiac puncture with sterile disposable syringe, before dissecting the animals. Serum was separated by centrifugation at 2000 rpm for 15 min using BHG Hermle Z230 Centrifuge machine and the serum was carefully aspirated with a Pasteur pipette into sample bottles for the various biochemical assays. The rats were quickly dissected and whole liver and heart were excised, freed of fat, blotted with clean tissue paper, and then weighed. Assay Kits (Diagnostica Merck, Germany) were used for biochemical analysis.

The levels of cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and levels of alanine transaminase, aspartate transaminase and alkaline phosphatase in both the serum and tissues were determined spectrophotometrically by Hitachi U-2000 spectrophotometer on the same day [10].

Statistical Analysis

All data were statistically analysed with Students't-test and two-way ANOVA, followed by Tukey Krammer post-test. Values of $P < 0.05$ were considered significant.

RESULTS

Wistar rats intraperitoneally injected with alloxan revealed 3 folds increase in blood glucose levels as compared to control (non-diabetic) revealing hyperglycaemic or diabetic phase. Continuous oral administration of aqueous extract of mixture of *J. curcas* and *J. gossypiifolia* roots showed a significant ($P < 0.05$) reduction in blood glucose level from day 3 to day 15 as revealed in Table 1. The highest percentage reduction in the blood glucose level was obtained with the administration of the mixture

Table 1. Antihyperglycaemic activities of *J. curcas, J. gossypiifolia* and their mixture on Alloxan-induced Diabetic rats

	Group / Blood Glucose Level					
Day	**NDDW**	**DDW**	**DSD**	**DJc250**	**DJg250**	**DM250**
1	78.50	314.07	316.70	310.30	309.70	307.80
	% Change in Blood Glucose Level					
3	Not hyperglycaemic	1.49	23.97	5.09	16.14	39.70
5	Not hyperglycaemic	0.93	72.47	33.03	30.51	42.33
7	Not hyperglycaemic	27.77	74.58	37.32	66.58	62.30
9	Not hyperglycaemic	26.69	63.31	53.50	64.26	66.00
11	Not hyperglycaemic	3.24	66.91	44.03	58.25	65.10
13	Not hyperglycaemic	6.89	70.07	65.90	61.16	73.30
15	Not hyperglycaemic	29.55	69.98	65.94	48.11	69.60

n=6±SEM, Values with different superscripts along the column are significantly different (P<0.05)
NDDW = Non-diabetic + distilled water; DDW = Diabetic + distilled water; DSD = Diabetic + 14.2 mg/kg body weight standard drug; Dk250 = Diabetic + 250 mg/kg body wieght J. curcas; DJg250 = Diabetic + 250 mg/kg body wieght J. *gossypiifolia*; DM250 = Diabetic + 250 mg/kg body wieght Mixture.

Table 2 depicts the result of effects of mixture of roots of *J. curcas* and *J. gossypiifolia* on the hyperlipidaemic condition of the alloxan-induced Diabetic rats. The mixture significantly (*P<0.05*) reduced the concentrations of total lipids, triglyceride and low-density lipoproteins while the concentration of high density lipoproteins significantly (*P*<0.05) increased. Although *J. curcas* and *J. gossypiifolia* were able to reduce the serum total lipid to normal (NDDW) when compared with diabetic-distilled water group (DDW), there is no significant different (*P*<0.05) in the effect of either single or combined extract of *J. curcas* and *J. gossypiifolia*.

The serum levels of ALT, AST and ALP activities were significantly increased in the diabetic rat while in other tissues their activities reduced significantly (*P*<0.05). However, oral administration of the extract significantly (*P*<0.05) reduced and increased the activities of the enzymes in the serum and the tissues respectively as shown in Figures 1, 2 and 3.

Table 2. Effects of oral administration aqueous extracts (250 mg/kg body weight) of roots of *J. curcas, J. gossypiifolia* and mixture on serum lipid concentration (g/L) of alloxan-induced diabetic rats

Group	Total Lipids (mmol/L)	Triglyceride (mmol/L)	LDL (mg/dl)	HDL (mg/dl)
NDDW	4.01±0.24[a]	1.00 ±0.11[a]	0.38±0.02[a]	0.24±0.02[a]
DDW	7.07±0.39[b]	1.85±0.08[b]	0.76±0.04[b]	0.15 ±0.01[b]
DSD	4.15±0.43[a]	1.50±0.10[c]	0.43±0.02[c]	0.23 ±0.01[a]
DJc250	4.36±0.19[a]	1.65±0.06[c]	0.51±0.06[c]	0.21 ±0.01[a]
DJg250	4.41±0.12[a]	1.50±0.09[c]	0.51±0.04[c]	0.23±0.01[a]
DM250	4.18 ±0.22[a]	1.52 ±0.06[c]	0.45±0.04[c]	0.23 ±0.01[a]

n=6±SEM, Values with different superscripts along the column are significantly different (*P*<0.05)
NDDW = Non-diabetic + distilled water; DDW = Diabetic + distilled water; DSD = Diabetic + 14.2 mg/kg body weight standard drug; Dk250 = Diabetic + 250 mg/kg body wieght J. curcas; DJg250 = Diabetic + 250 mg/kg body wieght J. *gossypiifolia*; DM250 = Diabetic + 250 mg/kg body wieght Mixture.

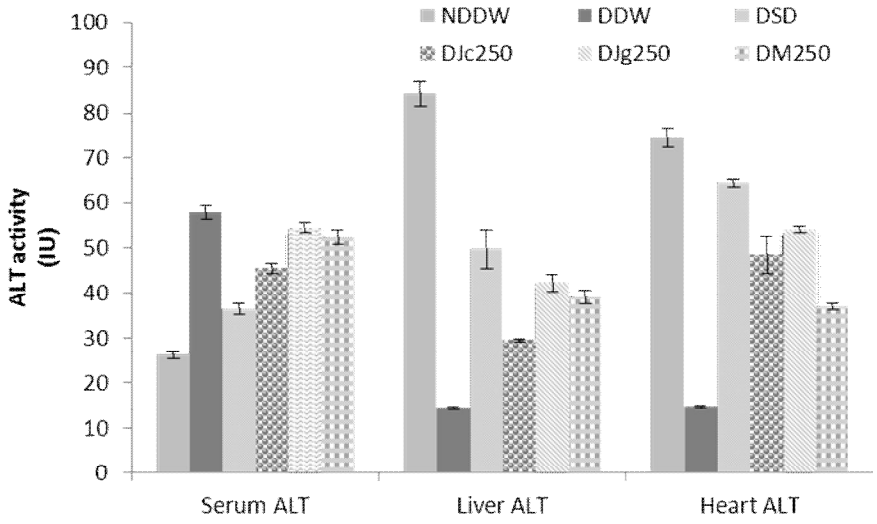

Figure 1. Effects of oral administration of aqueous extracts (250mg/kg body weight) of roots of *J. curcas, J. gossypiifolia* and Mixture on Alanine Transaminase (ALT) activity in selected tissues of alloxan-induced diabetic rats.

Values are expressed as Mean±S.E.M and bars with different letters are significantly different (*P*<0.05). NDDW = Non-diabetic + distilled water; DDW = Diabetic + distilled water; DSD = Diabetic + 14.2 mg/kg body weight standard drug; Dk250 = Diabetic + 250 mg/kg body wieght *J. curcas*; DJg250 = Diabetic + 250 mg/kg body wieght J. *gossypiifolia*; DM250 = Diabetic + 250 mg/kg body wieght mixture.

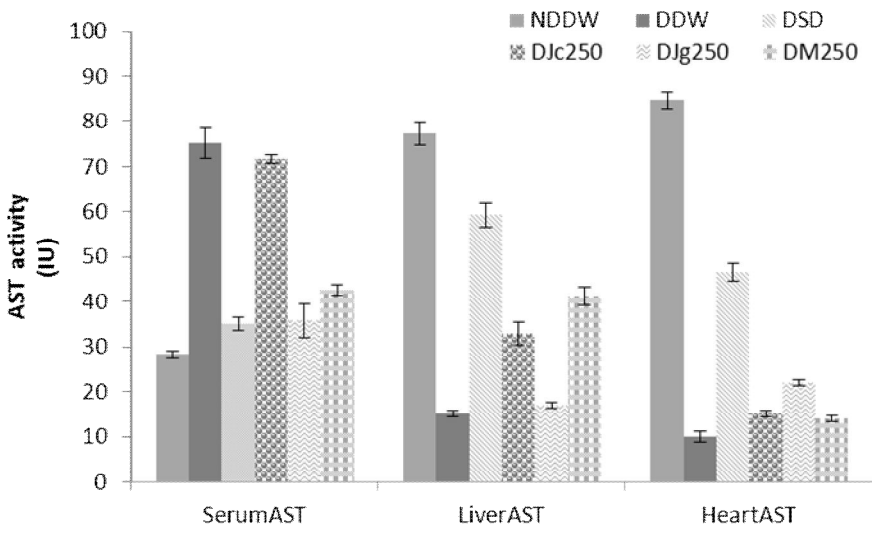

Figure 2. Effects of oral administration of aqueous extracts (250mg/kg b.wt) of root of *J. curcas, J. gossypiifolia* and mixture on aspartate transaminase (AST) activity in alloxan-induced diabetic rats

Values are expressed as Mean±S.E.M and bars with different letters are significantly different (P<0.05). NDDW = Non-diabetic + distilled water; DDW = Diabetic + distilled water; DSD = Diabetic + 14.2 mg/kg body weight standard drug; Dk250 = Diabetic + 250 mg/kg body wieght *J. curcas*; DJg250 = Diabetic + 250 mg/kg body wieght J. *gossypiifolia*; DM250 = Diabetic + 250 mg/kg body wieght mixture.

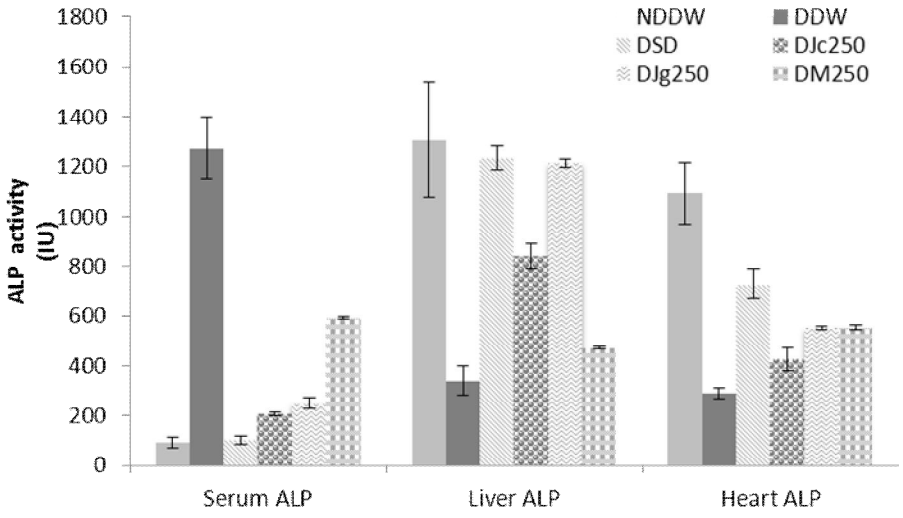

Figure 3. Effects of oral administration of aqueous extracts (250mg/kg b.wt) of root of *J. curcas, J. gossypiifolia* and mixture on alkaline phophatase (ALP) activity in alloxan-induced diabetic rats

Values are expressed as Mean±S.E.M and bars with different letters are significantly different (P<0.05). NDDW = Non-diabetic + distilled water; DDW = Diabetic + distilled water; DSD = Diabetic + 14.2 mg/kg body weight standard drug; Dk250 = Diabetic + 250 mg/kg body wieght *J. curcas*; DJg250 = Diabetic + 250 mg/kg body wieght J. *gossypiifolia*; DM250 = Diabetic + 250 mg/kg body wieght Mixture.

DISCUSSION

One of the major diseases that usually claim many lives all over the world is diabetes [11]. Reduced concentration of circulating insulin, poor insulin sensitivity or insulin resistant characterizes diabetic state. A typical diabetes condition may result in hyperglycaemic state of diabetic subjects with symptoms of frequent urination (polyuria), passage of urine with glucose (glucosuria), frequent hunger (polyphagia) among others. Using oral hypoglycaemic agents for the management of

diabetes disease is now common especially for the type II diabetes. Such agents include sulphonylureas and biguanides, despite the report on their negative effects. Therefore, in searching for alternative agent that will be cheaper and safer, attention has now been focused on the use of medicinal plants for the management of diabetes disease ([12-14].

The result from the oral administration of the extracts of *J. curcas, J. gossipiifolia* and their mixture clearly demonstrates that the combined extract exerts significant anti-hyperglycaemic effects in the alloxan-induced diabetic rats. Although *J. gossypiifolia* is more effective as antihyperglycaemic agent than *J. curcas*, the combined extract is more effective than a single extract. Therefore, if the combined extract is characterized to isolate the active component it could serve as a more effective, accessible and affordable alternative to orthodox antidiabetic.

Insulin deficiencies also affects lipid metabolism leading to lipolysis (mobilization of free fatty acids from the peripheral fat depots) and high cholesterol levels culminating in low concentrations of HDL with high concentrations of LDL, triglycerides and total lipids. Diabetes mellitus also affects lipid concentration [15]. Induction of diabetics increased the triglyceride and LDL concentrations while the HDL level was reduced. All the animal groups treated with either single or combined extract had reduced level of triglyceride.

The increased LDL level in alloxan-induced diabetic rats (DDW) was reduced when treated with either single or combined extracts while the HDL concentration was increased. Therefore, normalization of the concentration of serum lipid concentration in a diabetic individual could be achieved using either single or combined extract of *J. curcas* and *J. gossypiifolia*.

Diabetes mellitus also affects some enzymes especially in the liver [16]. The measurement of the activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation, diagnosis and tissue cellular damage [17]. Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked up by convectional histological techniques [18]. ALP activity has been the marker enzyme for plasma membrane [19] and is required in certain amounts for proper functioning of organs. The reduction in the activity of the liver ALP observed in this study may be due to damage to the plasma membrane leading to loss of this enzyme into the extracellular fluid [20]. This was reflected with the increase in the serum ALP. Other enzymes such as the transaminases occupy a central position in the metabolism of amino acids. The increase in serum ALT and ASP levels in diabetic rats could be related to excessive accumulation of amino acids (alanine and glutamate) in the serum of diabetic animals because of amino acids mobilization from protein stores for which the enzymes ALT and ASP are needed, leading to increased activities [21] or leakages from the tissues [18]. From the results of this study, an increase in serum level of these enzymes may be due to leakages from the tissues since there is a corresponding decrease in the enzyme activities at the tissue levels.

CONCLUSION

Individuals with diabetes mellitus are likely to have abnormal blood glucose level, higher lipid concentration and decreased activities of some liver function biomarker enzymes. In the management of diabetes mellitus, *J. curcas* and *J. gossypiifolia* could be very good candidates as demonstrated in this study where either single or combined extract was able to ameliorate the disease. Although, either of *J. curcas* and *J. gossypiifolia* are good antidiabetes agent as shown in this research, the combined extract of the two plants is more effective for the treatment of diabetes mellitus.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Professor Nasir Olanrewaju Muhammad (of blessed memory) for his immense contribution to the success of this research. The authors declared that no conflicts of interest with respect to this research, authorship, and/or publication of this article.

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