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Chemical composition and anti-diabetic properties of *Jatropha curcas* leaves extract on alloxan induced diabetic wistar rats

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This study evaluates the chemical composition and anti-diabetic properties of fresh and shade dried *Jatropha curcas* aqueous leaves extracts (JCLE) on alloxan induced diabetic female wistar rats. Seven (7) kg of *J. curcas* leaves were pulverized and aqueous extracts produced. Thirty five (35) mature female rats were grouped into seven of five animals per group according to their body weights. Blood samples were collected for baseline data before inducing diabetes. Various groups of rats were fed graded doses (100, 200 and 300 mg of JCLE, respectively. Blood glucose was tested every seven days using glucometer. The animals were treated for 21 days with JCLE, blood samples were collected for liver enzyme function test, liver and pancreas tissues collected for histopathology. The results obtained were analysed statistically using analysis of variance (ANOVA) and Duncan Multiple range student test. Result shows that proximate composition of JCLE had higher protein, fat and carbohydrate concentrations in shade dried than the fresh samples. The vitamin, mineral and phytochemical compositions varied but higher in shade dried JCLE also than the fresh. Rats treated had significant ($p<0.05$) reduction in blood glucose level. Liver enzymes was higher with shade dried JCLE. The current study provides some useful insight into the anti-hyperglycemic potency of JCLE in alloxan induced diabetics and seems to repair some organs damages.

Key words: Blood glucose, *Jatropha curcus,* liver enzymes, diabetic.

INTRODUCTION

In Sub-Saharan Africa, there is a growing need to fight the scourge of diabetes among the populace. Although access to primary health care is low, diabetic patients tend to fight this disease through natures endowed plant resources. Prevalence and burden of type 2 diabetes are rising quickly and may be due to the rapid uncontrolled urbanization, environmental degradation and major changes in lifestyle of the people (Jean et al., 2010;

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Abbreviations: JCLE, Jatropha curcas aqueous leaves extracts; **AST,** Aspartate aminotransferase.

Mohan et al., 2013). This increase presents a substantial public health and socioeconomic burden in the face of scarce resources. Therefore, there is a concomitant increase in alternative but cheaper means of treating this disease condition among the rural and low economic income earners. In 2003, the International Diabetes Federation estimated that approximately 194 million people around the world had diabetes and by 2025 this figure will be expected to rise to 333 million, amounting to 6.3% of the world's population living with diabetes (Atlas, 2003). The Diabetes Association of Nigeria (2011) also reported that over 140 million people based on Nigerian Census of 2006, had an estimated six million persons with full blown diabetes mellitus. Reports from hospital records indicate the alarming increase in both prevalence and incidence of diabetes among all ethnic groups and social classes in Nigeria (DAN, 2011). Indeed, diabetes has in the last two decades become a household disease that requires urgent health care intervention.

Jatropha curcas is a drought resistant shrub or tree belonging to the family Euphorbiaceae, which is cultivated in Central and South America, South-East Asia, India and Africa (Martinez-Herrera et al., 2006). Recently, there is an increasing use of *J. curcas* in the management of diabetes and other carbohydrate metabolic related syndromes. Other ethnomedical uses of the extracts of *J. curcas* leaves include use as a remedy for cancer, as an abortifacient, antiseptic, diuretic, purgative and haemostatic (Dalziel,1995; Mishra et al., 2010). *J. curcas* leaves have been observed to be consumed by people with many ailments traditionally including diabetes mellitus in the North-east of Nigeria without scientific explanation. Thus, the main objective of this work was to assess the chemical, phytochemical and anti-diabetic properties of *J. curcas* leaves extract.

MATERIALS AND METHODS

Samples preparation

The *J. curcas* leaves were harvested, sorted by removing extraneous materials; the sample leaves were then taken to the taxonomic unit in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka for identification. The leaves were rinsed with distilled water and divided into two parts; 5 kg fresh and 5 kg was shade dried for 5 days at room temperature. The dried leaves were pulverized into powder using Warburg laboratory blender and packaged in labelled polyethylene bags for further use.

Preparation of vegetable extracts for rat studies

Two hundred grammes (200 g) each of the pulverized vegetable samples were soaked separately in 500 ml boiled distilled water and agitated intermittently for 24 h. They were filtered using fine sieve of 4.75 mm mesh (No. 4) to obtain the aqueous extracts. The extracts were stored in air tight containers at 4°C and later reconstituted in distilled water to give the required doses to be administered during the study using the formula:

$$\frac{\text{Weight of rat x conc. of extracts}}{\text{Dosage x 1000}}$$

Chemical analysis

Proximate analysis

Portions of *J. curcas* leaves were analysed for their proximate values using AOAC methods (2005).

Moisture

The moisture content of the samples was determined using the hot oven method as described by Pearson (1976). Petri dishes were thoroughly washed and dried in the oven at 100°C for 30 min and allowed to cool inside a desiccator. These various weights were determined by weighing them with a weighing balance. 2 g of finely ground sample was weighed into the dishes and placed inside the oven at 100°C for 4 h. The dishes together with the sample they contain were removed, cooled in a desiccator and weighed. They were placed back into the oven, dried for further 30 min, cooled and weighed. The drying was continued and weighed repeatedly until a constant weight was obtained. The percentage moisture content was calculated from the weight loss of the sample.

Thus:

$$\% \text{ moisture} = \frac{\text{Wt. of dish + sample – wt. of dish + sample after drying}}{\text{Wt. of sample}} \times \frac{100}{1}$$

Protein

Total nitrogen was estimated using micro kjeidahl method as described by Pearson (1976). One gram of sample was digested with concentrated sulphuric acid. The digested samples were distilled and titrated. The crude protein was calculated by multiplying the total nitrogen by the conversion factor of 6 to 25.

$$P = TN \times 6.25$$

Fat

The fat content of the samples were determined using Soxhlet extraction method as described by Pearson (1976). The fat content was determined using Soxhlet fat extraction unit/system. The Soxhlet extraction cup was washed and dried in oven and weighed in an analytical balance. One gram sample was weighed and placed in a folded filter paper inside the thimble. The thimble was immersed into an aluminium cup containing 30 ml of petroleum ether. It was further placed in the extraction unit and the extraction process began. After the extraction process the aluminium cup together with the oil it contains was sent to the oven for drying before weighing.

$$\% \text{ of wt.} = \frac{A - B}{C} \times \frac{100}{1}$$

Where, A = weight of empty cup; B = weight of cup + fat; and C = weight of sample used in grams.

Carbohydrate

Carbohydrate This was determined by difference
% carbohydrate = 100 - (% ash + protein + fat + fibre + moisture).

Ash

Ashing method by Osborne and Voogt (1973) was used. Two grams each of the samples was subjected to ashing, in a silica crucible until the food matrix was destroyed. The sample was heated gently at first at about 1500C to clay it and at 500°C in a muffle furnace to completely destroy it.

$$\% \text{ Ash} = \frac{C - B}{F} \times \frac{100}{1}$$

Where, B = weight of crucible (empty); C = weight of crucible + Ash; and F = weight in grams of sample used.

Crude fibre

The fibre content was determined using the AOAC method (1995). Three grams of sample was defatted by Soxhlet extraction or by stirring, setting and decanting with petroleum ether. The defatted sample was placed in a beaker with 200 ml of boiling H_2SO_4. It was filtered under suction, washed with distilled water and boiled again for another 30 min with 200 ml of NaOH. The digested sample was washed with 0.1 m HCl to neutralize the NaOH and severally with hot distilled water. It was washed 2 times with ethanol and three times with ether. The ash is then cooled and weighed.

$$\% \text{ fibre} = \frac{M_2 - M_3}{M0} \times 100$$

Where, M_2 = weight after drying; M_3 = weight after ignition; M_0 = weight of sample.

VITAMINS

Vitamin A (βeta carotene)

The method of AOAC (2005) was used. One gram of the sample was extracted with 50 ml of petroleum ether in triplicate. The ether extract was concentrated and evaporated to dryness. The residue was dissolved with 0.2 ml of chloroform-acetic anhydride (1:1). 2 ml of trichloroacetic acid-chloroform (1:1) was added and the absorbance taken at 620 nm every 15 s interval.

Vitamin B1 (Thiamine)

One (1) ml of the filtrate was transferred into three test tubes, 2 ml of water; 0.4 ml of 50% sodium acetate, 0.1 ml of diazotized reagent was added and shaken. 0.2 ml of 5.5% sodium carbonate was added, mixed and the absorbance taken at 540 nm against a reagent blank.

Mineral analysis

Calcium

Two grams of sample was ashed followed by classical precipitation and titration (Paul and Southgate, 1978).

Iron and zinc

The method of AOAC method (1995) was used. Two grams of sample (powdered) is weighed into a crucible and ashed in a muffle furnace at 550°C for 6 h. The ash was cooled and 6 HCl added and boiled for 10 min, while covering the crucible with a watch glass. After boiling, the sample was cooled and filtered into 100 ml volumetric flask. The crucible washed with distilled water and the washing added to ash filtrate. The ash filtrate was made to 100 ml with distilled water.

An aliquot of the filtrate was aspirated into the atomic absorption spectrophotometer (Pye Unicam) and the absorbance values corresponding to the different minerals read. Solutions of Zn and Fe were prepared and aspirated into the atomic absorption spectrophotometer and their absorbance values recorded. The percentage of element present was calculated from the absorbance values of the sample and standard solution.

Potassium

Potassium content of the samples was determined using the atomic absorption spectrometric method as described by Collins and Polkinhorne (1952). 5 g of the sample was ashed, then ash was transferred to 400 ml beaker using 100 ml distilled water. About 10 ml concentrated of HCl was added and boiled for several minutes then cooled and diluted with water to 500 ml and filtered, diluted to final conc. of solution (approximately 15 mg/L K_2O) prepared. A series of solution from the freshly prepared diluted potassium solution containing 10, 12, 14, 16, 18 and 20 mg/l K_2O was read against blank (the concentration was read between 766 to 770 nm in an atomic absorption spectrophotometry (AAS). Results were extrapolated from an already prepared calibration curve.

$K = 0.88 \times k_20$

Phosphate

The method of AOAC (2005) was used. 2 ml of sample was transferred into three test tubes and 3 ml of water added; the pH was adjusted to 7.0 with dilute ammonia and 2.5 ml of vanadate molybdate reagent added. The solution was made up with 10 ml and after 10 min the absorbance was taken at 470 nm against a blank.

Experimental design

Toxicity test

The acute toxicity and mean lethal dose (LD_{50}) of the extracts were determined in mice using the method described by Lorke (1983) (Table 1b). The experimental animals were healthy female Wistar rats weighing between 150 to 200 g. Thirty five rats were randomly divided into seven groups (1 to 7) of five rats each (Table 1a). These rats were distributed in metabolic cages and maintained under standard environmental conditions. They were fed with commercial rat chow and water *ad libitum*. Mature female Wistar rats were procured from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, for this study. The test sample *J. curcas* leaves were obtained from No. 57 Ibagwa road, Nsukka, Nigeria.

Ethical consent

Ethical consent was obtained from the Ministry of Veterinary Control Enugu, Enugu state Nigeria.

Induction of diabetes

Diabetes mellitus was induced in groups' 1-6 rats by single intra-

Table 1a. Grouping of Wistar rats administered graded doses of *J. curcas*.

Group	Sample	Dose (mg/kg bwt)
1	Shade dried *J. curcas*	100
2	Shade dried *J. curcas*	200
3	Shade dried *J. curcas*	300
4	Fresh *J. curcas*	100
5	Fresh *J. curcas*	200
6	Fresh *J. curcas*	300
7	Control	

Table 1b. LD_{50} value of the aqueous extract of *J. curcas* leaves.

No. of mice	Dose (mg/kg)	No. of death of animals
3	5	0
3	50	0
3	300	0
3	2000	1
3	3000	3

LD_{50} value = 2000 mg/kg, animals used = albino mice, weight of animals = 20-25 g. No. of animals = 3, Route = oral.

peritonial injection of 150 mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, USA) suspended in normal saline, after an overnight fasting on the 5th day. After 48h (7th day), diabetes was confirmed using one touch glucometer. Animals that had fasting blood glucose level ≥200 mg/dl were considered diabetic and included in the study according to Luka and Tijjani (2013).

Collection of blood samples

Blood samples were collected from the retro-bulba plexus of the medial canthus of the eye of the rat for biochemical analysis.

Blood glucose estimation

Blood glucose was estimated on day 0, 7, 14 and 21 using glucometer (One Basic, Inc.).

Assay of activities of some liver function enzymes

Serum enzymes; aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined using Randox laboratory reagent kit, UK, BT 29 4QY based on method by Reitman and Frankel (1957).

Histopathology examination

At the end of the experiment, the rats were anesthetized and dissected. The liver and the pancreas tissues were extracted, rinsed in normal saline for histopathological examination after which they were fixed in 10% formalin. Thereafter, the tissues were cleared in chloroform overnight, infiltrated and embedded in molten paraffin wax. The blocks were later trimmed and sectioned at 5 to 6 microns. The sections were deparaffinized in xylene, taken to water and subsequently stained with Haematoxylin and Eosin (H and E) for light microscopy (Bancroft and Stevens, 1977).

Statistical analysis

Data obtained were analyzed using descriptive statistics including mean and standard deviation. Duncan new multiple range test and least significant difference (LSD) were used to separate the means. Significant differences were accepted at $p< 0.05$.

RESULTS AND DISCUSSIONS

Acute toxicity study (Table 1b) is generally carried out for the determination of LD_{50} value in experimental animals. The LD_{50} determination was done in mice by OECD guidelines 423. The aim of performing acute toxicity study was for establishing the therapeutic index of a particular treatment and to ensure the safety *in vivo*. The LD_{50} of the 50% aqueous extract of *J. curcas* was found to be 2500 mg/kg. The proximate composition of shade dried and fresh *J. curcas* leaves extracts (JCLE) as displayed in Table 2 revealed that shade dried samples contained higher percentage of protein, ash, fat and carbohydrate as compared with the fresh. However, the fresh JCLE had higher percentage of moisture content (94.12%) as compared with the shade dried leaves extract (90.00%). The lower moisture content observed in the shade dried JCLE could possibly be due to loss of moisture during the shade drying periods. Fresh vegetables are known to contain more water than either sun or shade dried vegetables. Udofia and Obizoba (2005) reported the same observation in fresh 'attama and sweet potato leaves. The protein content (1.90 and 2.01%) of fresh and dried JCLE were lower than 3.3% recorded by USDA Nutrient Database for Standard Reference (Hall, 1998). This may be because some protein fractions may still be embedded in the leave samples. Chima and Igyor (2007) also reported lower protein values for (*Pterocarpuss oyauxii*) "Oha" (2.0%) and *Gnetum africanum* "Okazi" (1.5%). Incorporating *J. curcas* vegetable in diet can furnish it with appreciable amount of protein which provides enormous benefits such as maintenance of fluid balance, formation of hormones and enzymes and contribution to immune function. Ash, carbohydrate and fat were generally lower than other authors who reported *Ipomea batata* (11.10%*)* and *Moringa oleifera* (15.09% DW) (Antia et al., 2006; Lockeett et al., 2000) and *Hibscus esculentus* (8.00% DW) reported by Akindahunsi and Salawu (2005). Ash content which is an index of mineral contents in biota was 0.69 and 0.98%, respectively, for fresh and dried JCL. Carbohydrate value for dried leaves was 6.04% lower than that observed by Akubugwo et al. (2007) in *Amaranthus hybrids* (52.18%) fresh samples, but higher than *Thuja occidentalis (*4.72%*).* As far as vegetables are concerned, some of them are rich sources while others contain traces of some the nutrients.

Table 3 shows the vitamin composition of shade dry and

Table 2. Proximate composition of shade dry and fresh *Jatropha curcas* aqueous leaves extract.

Sample	Protein (%)	Ash (%)	Fibre (%)	Fat (%)	Moisture (%)	CHO
Shade dry	2.01±0.03	0.98±0.03	-	0.97±0.04	90.00±0.33	6.04±0.30
Fresh	1.90±0.06	0.69±0.10	-	0.55±0.05	94.12±1.16	2.73±1.09

Mean ±SD of three determinations.

Table 3. Vitamin composition of shade dry and fresh *Jatropha curcas* leaves extract

Sample	Pro-vitamin A (IU)	Vitamin C (mg/100 g)	Vitamin B1 (mg/100 g)	Vitamin B2 (mg/100 g)	Vitamin B3 (mg/100 g)
Shade dry	1491.30±28.48	0.98±0.03	90.00±0.33	6.04±0.30	10.00±0.33
Fresh	1312.80±46.9	0.65±0.11	4.40±0.87	0.07±0.01	2.63±0.15

Mean ±SD of three determinations.

Table 4. Mineral composition of shade dry and fresh *Jatropha curcas* aqueous leaves extract.

Sample	Iron (mg/100 g)	Calcium (mg/100 g)	Potassium (mg/100 g)	Zinc (mg/100 g)
Shade dry	0.33±0.0	0.74±0.06	63.81±0.32	42.33±2.52
Fresh	0.60±0.02	0.49±0.01	76.45±1.77	32.00±2.00

Mean ±SD of three determinations.

Table 5. Phytochemical composition of shade dry and fresh *Jatropha curcas aaqueous* leaves extract.

Sample	Alkaloid (mg/100 g)	Tannin (mg/100 g)	Oxalate (mg/100 g)	Flavonoids (mg/100 g)
Shade dry	2.26±0.24	1.63±0.32	8.60±0.60	3.83±0.21
Fresh	0.99±0.04	0.07±0.02	4.58±0.20	2.23±0.12

Mean ±SD of three determinants.

fresh *J. curcas* leaves extract. The result shows that the vitamin composition of shade dried JCLE was higher (vitamin B190.00 mg, Vitamin B26.04 mg and Vitamin B310.00 mg) than the fresh JCLE (vitamin B1 4.40 mg, Vitamin B2 0.07 mg and Vitamin B3 2.63 mg). Pro-vitamin A and vitamin C contents were also higher in the shade dried samples than the fresh samples. This is similar to the result of other researchers who reported higher vitamin contents in dried samples than the fresh samples (USDA, 2012; Reddy and Love, 1999). Generally, vegetables are rich in vitamin, minerals, dietary fibre and protein (Otitoju et al., 2014; Humphrey et al., 1993; Mathenge, 1997). The result in this study shows that JCLE is a rich source of pro-vitamin A and vitamin B. Pro-vitamin A protects the body cells from the damaging effects of free radicals, they act as good source of vitamin A and enhance the functioning of the immune system; it also helps the reproductive system to function properly (Handelman, 2001; Young and Lowe, 2001). Table 4 shows the mineral composition of shade dried and fresh *J. curcas* leaves extracts. The fresh samples had higher iron content (0.60 mg) than the dried samples (0.33 mg). A similar trend was observed for potassium (76.45 and 63.81 mg), respectively. In shade dried *J. carcus,* calcium (0.74 and 0.49 mg) and zinc (42.33 and 32.00 mg) were higher in the dried than the fresh samples, respectively. Quantitative phytochemical compositions result (Table 5) shows that shade dried samples had higher alkaloids (2.26 mg), tannins (1.63 mg), oxalate (8.60 mg) and flavonoid (3.83 mg), respectively than the fresh samples which contained alkaloid (0.99 mg), tannins (0.07 mg), oxalate (4.58 mg) and flavonoid (2.23 mg), respectively. Oladele et al. (1995) have also reported that medicinal plants with hypoglycemic and anti-diabetic effect usually contain high concentration of alkaloids and flavonoids. This finding is in line with the result obtained from this study.

This study demonstrates hypoglycaemic effect on the fasting blood glucose level of the rats (Table 6) and had significant ($p < 0.05$) increase in the blood glucose levels after induction with 150 mg/kg alloxan. The highest percentage (70.68%) reduction of the blood glucose

Table 6. Effects of aqueous leaves extract of *J. curcas* (JCLE) on blood glucose levels in alloxan induced diabetic rats.

Group	Baseline (mg/	After induction (mg/dl)	After treatment (mg/dl)
1	87.00±6.32[a]	222.60±143.15[a]	112.33±1.53[a]
2	87.60±9.21[a]	374.0±163.21[b]	177.67±118.25[a]
3	93.40±7.79[a]	383.8±185.86[b]	112.50±6.36[a]
4	75.80±8.44[a]	313.8±198.09[b]	125.80±82.65[a]
5	67.80±4.66[a]	429.0±203.75[b]	155.25±139.09[a]
6	82.60±8.82[a]	350.4±216.47[b]	131.60±117.62[a]
7	81.60±1345[a]	75.0±7.42[a]	75.0±13.34[a]
LSD	0.002	0.056	0.80

Mean ± SD Mean values of different superscripts in the same row and significant at $p < 0.05$.

Table 7. Effect of aqueous leaves extract of *J. curcas* on aspartate amino transferase (AST) activity (IU/L) in alloxan induced diabetic rats.

Group	Baseline	After induction	After treatment
1	88.40±6.69[a]	120.80±12.85[b]	118.67±4.16[b]
2	83.0±11.27[a]	112.0±8.28[b]	105.33±9.45[b]
3	93.20±6.06[a]	108.20±7.95[b]	101.00±970[ab]
4	88.60±6.39[a]	119.40±15.47[b]	84.60±9.10[a]
5	89.0±5.96[a]	116.40±3.65[b]	89.40±3.13[a]
6	84.60±3.85[a]	109.40±9.86[b]	89.40±3.13[a]
7	95.60±4.04a	96.80±7.92 [b]	91.60±7.83a
LSD	0.077	0.014	0.000

Mean ±SD. Mean values of different superscripts in the same row and significant at $p < 0.05$.

levels was observed with the administrations of shade dried JCLE dose (300 mg) and for fresh (63.81%) with dose (200 mg) after-treatment compared with after-induction period. Similar results of hypoglycaemic effects of *J. curcas leaves* have been reported by Mishra et al. (2010) and Omale et al. (2011). Though there was significant decrease in the blood glucose level after-treatment as compared with the after-induction period, the blood glucose level was significantly ($p<0.05$) higher than the baseline blood glucose level. However, the shade dried and fresh aqueous extracts could not reverse the high blood glucose level observed in after-treatment to the initial baseline blood glucose level as reported by Mishra et al. (2010) in their work; this may be attributed to the use of the 50% ethanol extract while this study used aqueous extract for their treatments. It may also be a proof that the ethanol extract may be more effective as antidiabetic agent than the aqueous extract. Table 7 shows the mean aspartate aminotransferase (AST) level of the rats. The activity of AST after-treatment was significantly ($p < 0.05$) higher than the baseline values and the control group (group 7). Aspartate amino-transferase (AST) is an enzyme produced by the liver cells, elevated amounts of serum AST may signal toxicity which may lead to health problems. The levels of AST in the body are indicative of tissue damage and diseases and the amount released is proportional to the level of damage sustained (Evelson et al., 2005) increased with liver diseases in all species.

A number of other plants have been reported to have anti-hyperglycemic and insulin stimulatory effects (Latha and Pari, 2003; Latha and Pari, 2004; Pari and Venkateswaran, 2002). Increase in the activities of serum AST, ALT, and ALP may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (El-Demerdash and Abou, 2005), which gives an indication of the hepatotoxic effect of *J. curcas*. The levels of AST, ALT and ALP have been reported to increase in alloxan-induced diabetic rats (Nnodim et al., 2012). Both shade dried and fresh aqueous extract of *J. curcas* caused significant increase ($P<0.05$) in the level of some serum AST, ALT and ALP values after-treatment as compared with after-induction period as shown in Tables 7 to 9. However, though both the shade dried and fresh aqueous extract of *J. curcas* caused significant decrease ($p<0.05$) in the level of serum AST, ALT and ALP after-treatment when compared with after-induction, the levels of AST, ALT and ALP in the serum for the groups fed with shade dried extract were significantly ($p<0.05$) higher than the baseline values. These significantly ($p<0.05$) higher AST, ALT and ALP values obtained in after-treatment as compared with the baseline values could imply that the shade dried *J. curcas* extract had not completely revert induced diabetes in the experimental rats. Similarly, it could also imply that the hepato-protective effects of JCLE worked more in the groups treated with fresh leaves extracts than the groups treated with the shade dried leaf extract. Thus, it could be said that the fresh leaves extracts had more hepato-protective potentials than the shade dried leaves extracts.

Although, there was no significant difference in the weight of the rats following induction of diabetes and

Table 8. Effects of aqueous leaves extract of *J. curcas* on liver Alanine aminotransferase (ALT) level (IU/L) in alloxan induced diabetic rats.

Group	Baseline	After induction	After treatment
1	65.40±3.58[a]	72.20±6.18[a]	66.67±6.11
2	68.00±4.74[a]	74.00±8.09 [a]	94.67±3.06[b]
3	66.00±3.00[a]	70.40±1.52[b]	88.00±2.83[c]
4	72.40±4.83[a]	68.80±3.27	69.20±11.43[a]
5	65.00±3.16[a]	73.20±4.32[b]	65.75±4.50[a]
6	65.00±4.18	63.60±1.82[a]	64.80±4.66[a]
7	66.20±4.44	65.40±5.46[a]	59.60±`6.43[a]
LSD	0.08	0.015	0.00

Mean ±SD Mean values of different superscripts in the same row and significant at $p < 0.05$.

Table 9. Effects of aqueous leaves extract of *J. curcas* on liver alkaline phosphatase (ALP) in alloxan induced diabetic rats.

Group	Baseline	After induction	After treatment
1	42.40±5.77[a]	50.60±6.54[a]	76.0±2.65[b]
2	47.60±4.39[a]	51.40±5.03[a]	62.33±5.86[b]
3	53.0±4.90[a]	56.60±4.04[a]	55.50±6.36[a]
4	48.60±6.50[a]	48.80±6.89[a]	44.60±4.77[a]
5	45.80±3.49[b]	53.80±4.49[c]	37.50±5.26[a]
6	39.20±2.39[a]	63.80±3.11[b]	42.00±3.16[a]
7	44.0±3.16[b]	38.80±3.56[a]	42.20±1.79[ab]
LSD	0.002	0.00	0.00

Mean ±SD Mean values of different superscripts in the same row are significant at $p < 0.05$.

Table 10. Effects ofaqueous leaves extract of *J. curcas* onbody weight of diabetic rats.

Group	Baseline	After induction	After treatment
1	234.20± 49.77[a]	218.00±41.43[a]	230.33±30.67[a]
2	200.40±29.70[a]	188.80±29.12[a]	210.67±31.56[a]
3	192.60±47.83[a]	169.00±12.73[a]	194.00±11.31[a]
4	166.80±19.69[a]	160.60±14.72[a]	152.00±13.71[a]
5	178.80±40.56[a]	150.40±33.25[a]	181.50±45.53[a]
6	146.80±31.10[a]	146.00±28.87[a]	156.00±25.79[a]
7	124.75±17.46[b]	157.20±20.83[b]	164.60±13.85[b]
LSD	0.051	0.076	0.074

Mean values in the same row of different superscripts on the row are significant at $p < 0.05$.

after-treatment, untreated diabetic rats had lower body weight when compared to the normal and treated groups (Table 10). This is in line with some studies that reported significant weight reduction in untreated diabetic rats (Longe and Momoh, 2014). The histopathology result shows that the protective nature of the extract was dose dependent as there was still a clear evidence of liver cell damage in the group treated with 100 mg of shade dried leaves extract (Figure 1a). While Figure 1b showed regeneration process of the liver, a similar effect was also observed in the pancreas (Figure 1c and d). There was mild areas of vacuolations in the 200 mg treated group (Figure 1e) when compared with Figure 1f which showed normal pancreatic cell (control group).

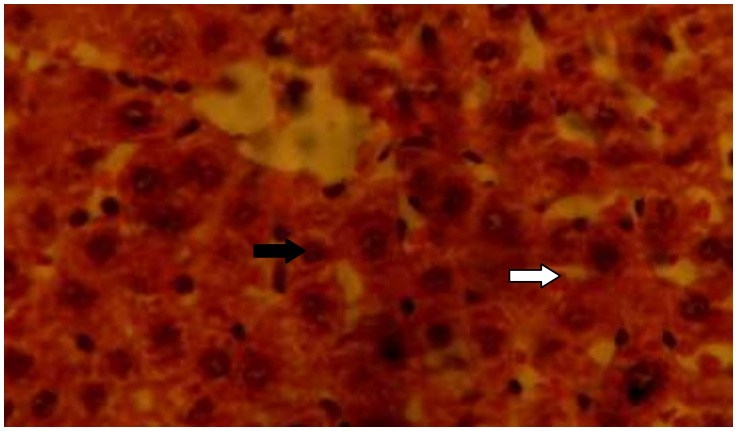

Figure 1a. Photomicrograph of liver section from group 1 (diabetic rats treated with 100 mg/kg dry leaf extract) showing mild hepatocellular degeneration (black arrow) and dilated sinusoids (white arrow). H and E x 400.

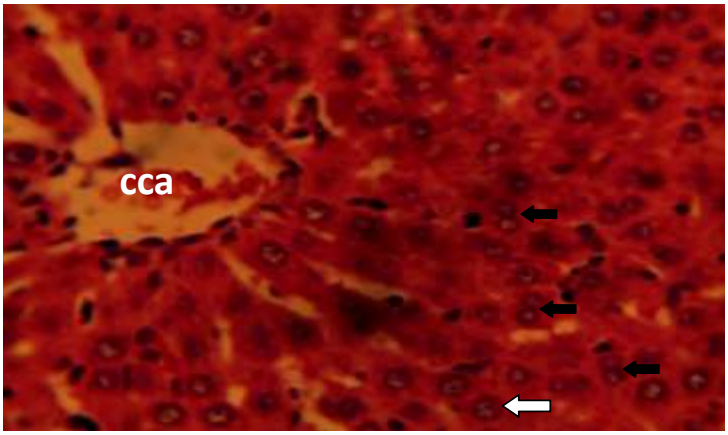

Figure 1b. Photomicrograph of liver section from group 6 (diabetic rats treated with 300 mg/kg fresh leaf extract) showing apparently normal hepatocytes (white arrows) and some binucleated hepatocytes-sign of liver regeneration (black arrows). H and E x 40.

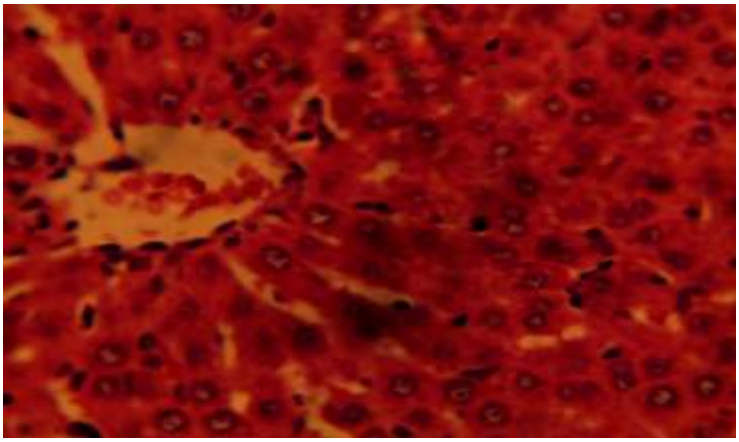

Figure 1c. Photomicrograph of liver section from group 3 (Control) showing apparently normal hepatocytes. H and E x 40).

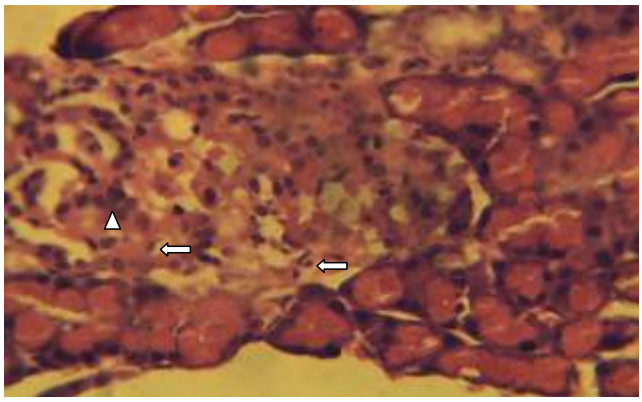

Figure 1d. Photomicrograph of sections of the pancreas from group 1 (diabetic rats treated with 100 mg/kg dry leaf extract) showing mild areas of vacuolations (arrows) and focal area of lymphocytic infiltration (arrowhead). H and E x 400.

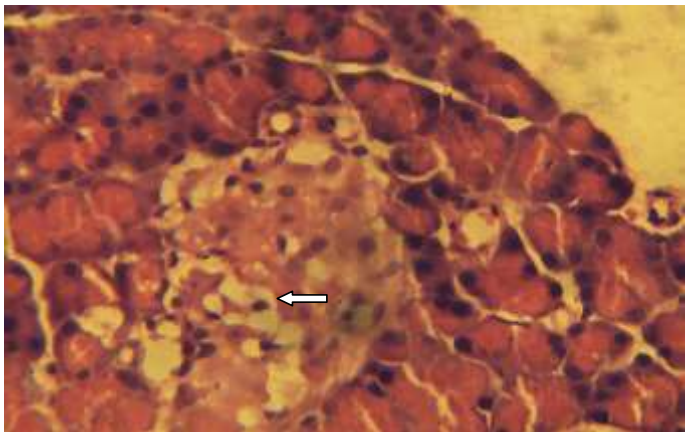

Figure 1e. Photomicrograph of sections of the pancreas from group 2 (diabetic rats treated with 200 mg/kg dry leaf extract) showing mild areas of vacuolations (arrows). H and E x 400.

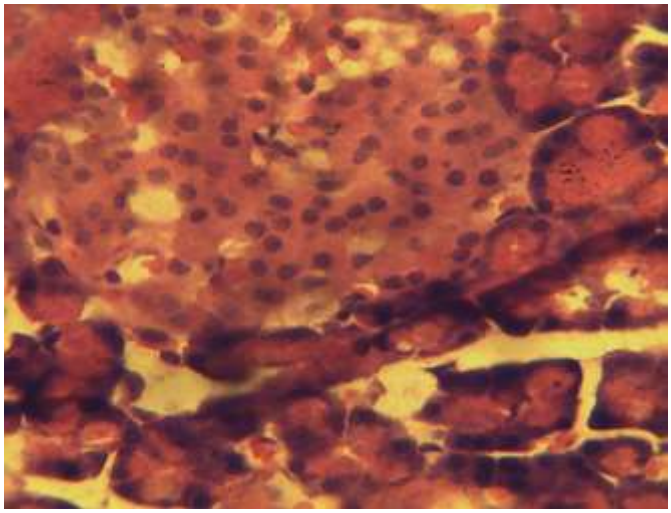

Figure 1f. Photomicrograph of pancreas section from group 5 (Control) showing apparently normal pancreatic cells. x 40.

Conclusion

The current study provides some useful insight into the antihyperglycemic potency of *J. curcas* leaves in alloxan induced diabetes. The shade dried and fresh aqueous extracts of *J. curcas* leaves used in this work showed strong anti-hyperglycemic effect as it significantly decreased the blood glucose level within the two weeks of administration. However, the shade dried and fresh aqueous extracts could not reverse the high blood glucose level observed after treatment to the initial baseline blood glucose level; this could be due to the aqueous extracts used or/and experimental duration (two weeks) at which the treatments were administered. In conclusion, *J. curcas* possess anti-diabetic property as already reported by other researchers but the aqueous plant extract may not be strong enough to revert completely diabetic condition under this experimental regime. Its mild toxicity is evident in the elevation of liver function enzyme (ALT and AST). However, some measure of processing may be required in order to reduce or eliminate its toxic potentials.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Akindahunsi AA, Salawu SO (2005). Phytochemical screening, nutrient and anti- nutrient composition of selected tropical green leafy vegetables. Afr. J. Biotechnol. 4(6): 497-501.

Akubugwo IE, Obasi NA, Chinyere GC, Ugbogu AE (2007). Nutritional and chemical value of *Amaranthus hybridus*L. Leaves from Nigeria. Afr. J. Biotechnol. 6(24):2833-2839

Antia BS, Akpan EJ, Okon PA, Umoren IU (2006). Nutritive and antinutritive evaluation of sweet potatoes (Ipomoea batatas) leaves. Pak. J. Nutr. 5:166-168.

AOAC (1995). Official methods of analysis of the association of official analytical chemists. Wasgington D.C. 16th Ed.

AOAC (2000). Official methods of analysis of the Association of Official Analytical Chemists, Washington D.C. 17th Ed.

AOAC (2005). Official methods of analysis Association on official Analytical chemists Washington D. C.

Atlas D (2003). Diabetes Atlas - International Diabetes Federation. International Diabetes Federation. First Published, *2000*. Second Edition, www.idf.org/sites/default/files/IDF_Diabetes_Atlas_2ndEd.pdf

Bancroft JD, Steven A (1977). Theory and Practice of Histological Techniques. Churchill Livingstone, New York, USA. pp. 88-89.

Chima CE, Igyor MA (2007). Micronutrient and anti-nutritional contents of selected tropical vegetables grown in South east, Nigeria. Nig. Food J. 25:111-116.

Collins GC, Polkinhorne H (1952). An investigation of anionic interference in the determination of small quantities of potassium and sodium with a new flame photometer. Analyst, 77: 430-436.

Dalziel JM (1995). The Useful Plants of West-Tropical Africa. London, Crown Agents for Oversea Governments and Administration, pp. 147

Diabetes Association of Nigeria (DAN) (2011). diabetesnigeria.org/Guidelines for diabetes management in Nigeria 2nd edition 2013 edited 17 -11- 2011.

El-Demerdash FM, Yousef MI, Abou El-Naga NI (2005). Biochemical study on the hypoglycemic effects of onion andgarlic in alloxan-induced diabetic rats. Food Chem. Toxicol. 43:57-63.

Evelson PC, Susemihl I, Villarreal S, Llesuy R, Rodríguez H, Peredo A, Lemberg J, Perazzo J, Filinger E (2005). Hepatic morphological changes and oxidative stress in chronic streptozotocin-diabetic rats. Ann. Hepatol. 4(2):115-120

Hall R (1998). Kale, *Brassica oleraceae* (Acephala Group).USDA Database for Standard Reference. about.com. Nutritionguide.http://www.nutrition.about.com. Accessed 06/11/2014.

Handelman GJ (2001). The evolving role of carotenoids in human biochemistry. Nutrition 17(10):818-22.

Humphrey CM, Clegg MS, Keen CL, Grivetti LE (1993). Food diversity and draught survival: The Hausa example. Int. J. Food Sci. Nutr. 44:1-16

Jean CNM, Ayesha AM, Eugene S, Felix KA, Sostanie TE (2010). Diabetes in sub-Saharan Africa. Lancet 375 (9733): 2254 – 2266.

Latha M, Pari L (2003). Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. Clin. Exp. Pharmacol. Physiol. 30:38-43.

Latha M, Pari L (2004.) Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some poly pathway enzymes in experimental rat diabetes. Braz. J. Med. Biol. Res. 37:577-586.

Lockeett CT, Calvert CC, Grivetti LE (2000). Energy and micronutrient composition of dietary and medical wild plants consumed during drought: Study of rural Fulani, Northeast Nigeria. Int. J. Food Sci. Nutr. 51:195-208.

Longe AO, Momoh J (2014). Effects of methanolic seed extract of *Hunteria umbellate* (abere) on blood glucose level, hematological and lipid profile parameters in alloxan- induced diabetes in male rats. Pinnacle Medicine & Medical Sciences. ISSN: 2360-9516.

Lorke D (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54:275-287

Luka C and Tijjani DH (2013). Comparative studies of the aqueous extracts of *Ocimum grtissimum, Aloe vera, Brassica oleracea* and *Ipomae abatata on* some biochemical parameters in diabetic rats. IOSR J. Pharm. Biol. Sci. 6(3):23-29.

Martinez-Herrera JP, Siddhuraju G, Francis G, Davila-Ortiz G, Becker K (2006). Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico. Food Chem. 96: 80.89.

Mathenge L (1997). Nutrition value and utilization of indigenous vegetables in Kenya. In: Guarino L. (Ed). Traditional African vegetables: Proceedings of the IPGRI International workshop on genetic resources of traditional vegetables in Africa. Conservation and use.

Mishra SB, Vijayakumar M, Ojha SK, Verma A (2010). Anti-diabetic effect of *jatrophacurcas*l. leaves extract in normal andalloxan-induced diabetic rats. Int. J. Pharm. Sci. 2(1):482-487.

Mohan V, Seedat YK, Pradeepa R (2013). The rising burden of diabetes and hypertension in Southeast Asian and African regions: Need for effective strategies for prevention and control in primary health care settings. Int. J. Hypertens. Article ID 409083, 14 pages. http://dx.doi.org/10.1155/2013/409083. Accessed 15/10/14

Nnodim J, Uduji HI, Uduji GO (2012). The role of *Gongronemalati folium* in attenuation of chloroquine induced. Glob. J. Biodivers. Sci. Manage. 2(1):43-46.

Oladele SM, Ayo JO, Adaudi AO (1995). Medicinal and physiological properties of flavonoids, coumarinderivativesandanthraquinones of plants origin . West Afr. J. Pharmacol. Drug Res. 11:134-144.

Omale J, Ebiloma GU, Agbaji AO (2011). Assessment of biological activities: A Comparison of Pergulariadaemia and Jatrophacurcas Leaf Extracts. Br. Biotechnol. J. 1(3):85-100.

Osborne DR, Voegt P (1973). The analysis of nutrients in food. Academic press Newyork, USA pp. 49-51.

Otitoju GTO, Nwamarah JU, Otitoju O, Iyeghe LU (2014). Nutrient composition of some lesser known green leafy vegetables in Nsukka LGA of Enugu State. J. Biodivers. Environ. Sci. 4(4):233-239.

Paul AA, Southgate DAT (1978). Mc Cance and Widdowson's The Composition of Foods, 4th ed.London:H.M. Stationery Office.

Pari L, Venkaleswaran S (2002). Hypoglycaemic activity of *Scoparia dulcis L.* Extract in alloxan induced hyperglycaemic rats. Phytother. Res. 16:662-664.

Pearson D (1976). Chemical analysis of food (7th edn), churchill, London.

Reddy MB, Love M (1999). The impact of food processing on the nutritional quality of vitamins and minerals. Adv. Exp. Med. Biol. 459:99-106.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28:56-63.

Udofia US, Obizoba IC (2005). Effects of sun and shade drying on nutrient and antinutrient content of some green leafy vegetables consumed in Uyo communities, Akwa Ibom State. J. Biomed. Investig. 3(1):1-5.

USDA (2012). Composition of Foods Raw, Processed, Prepared USDA National Nutrient Database for Standard Reference, Release 25: 1-128.

Young AJ, Lowe GM (2001). Antioxidant and proxidant properties of carotenoids. Arch. Biochem. Biophys. 385(1):20-27.