Anti-Diabetic Activities of the Leaf and Bark Extracts of Jatropha Curcas on Alloxan-Induced Diabetic Albino Rats

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Abstract

Antidiabetic activity was measured using a glucometer to check blood glucose level before induction with alloxan, after induction with alloxan and after treatment with both plant extracts. The animals were divided into seven groups, two of which were used for toxicological studies, three were used as control (negative, positive and normal) while the remaining two were used for the test groups. The extracts were found to exhibit hypoglycemic activity in the animals. The results of this study also revealed that the bark extract had more of the antidiabetic properties than the leaf extract as was observed in the change in blood glucose level of the animals in those groups. Histological studies was carried out on various organs of three of the groups among the seven groups (normal control group, group treated with leaf extract and group treated with the bark extract), it was observed that tissue necrosis was more prominent in the organs of the group treated with the leaf extract as compared to those treated with the bark extract when both were compared to the organs of the normal control group. The findings in this study provided the basis for further studies on the plant with the aim of finding out the mechanism of action of the folklore antidiabetic activity and the best extraction method of the toxic compounds without destroying other active components of the plant.

Keywords: *Jatropha curcas,* extracts, alloxan, diabetic.

Introduction

Diabetes mellitus is a disease characterized by an inability to regulate blood glucose which is caused by a relative or absolute deficiency in insulin. Insulin is a hormone secreted by the β-cells of the pancreas, and is required to utilize glucose from digested food as an energy source (Stong and Story, 2005). There is chronic hyperglycemia in diabetes and is associated with long term damage, dysfunction and failure of different organs, especially the eyes, kidney, nerves, heart and blood vessels (American Diabetes Association, 2012). Several pathogenic processes are involved in the development of Diabetes ranging from autoimmune destruction of the β-cells of pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action (American Diabetes Association, 2012).

Experimental Diabetes Mellitus has been induced in laboratory animals by several methods. Streptozotocin (STZ) 69% and Alloxan (31%) are by far the most frequently used drugs. Both drugs exert their diabetogenic action when administered parenterally, intravenously, intraperitoneally or subcutaneously (Etuk and Mohammed, 2010). The dose of these agents required for inducing Diabetes depends on the animal specials, route of administration and nutritional status (Balamurugan *et al*, 2003).

Streptozotocin prevents DNA synthesis in mammalian and bacterial cells. In bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA with eventual death of mammalian cells. Streptozotocin (STZ) prevents cecullar reproduction with a much smaller dose than the doze needed for inhibiting the substrate connection to the DNA or inhibiting any of the enzymes involves in DNA synthesis (Holam and Vanasscha, 2003).

Although, STZ is the most popular drug for induction of Diabetes in rats, there are some disadvantages to its use in chronic experiments especially spontaneous recovery from blood glucose level, by the development of functional insulin (Stemer *et al*, 1970) and high incidence of kidney and liver tumors. These problems are due strongly to the carcinogenic action of STZ (Anita *et al*, 2005).

Alloxan, a well-known diabetogenic agent is widely used to induce Type-2 Diabetes in animal (Bliss, 2000). Alloxan and its reduction product dialuric acid established a redox cycle with the formation of superoxide radical. These radical undergo dismutation to hydrogen peroxide. The action of reactive oxygen specie (R.O.S) with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B-cells (Chen and Wang, 2005). This alloxan induced Diabetes Mellitus serve as a pathological biomodel for testing a substance with supposed antioxidant activities in-vive (Choi, 2014).

Jatropha is a non-edible, oil seed plant from Euphabiacaea family. The plants of this family are an important source of medicine and toxins. The genus Jatropha is widely spread in the tropical regions of the world such as America, Africa and parts of Asian subcontinent. The word *Jetropha* is derived from a Greek word *Jatrus*

which means Doctors and *Trophe* which means Nutritional food. Jatropha is a genus of about 200 species that are succulent plants, shrub and trees. Since ages, the extract from different parts such as root, stem, bark and leaves of Jetropha plant have been used in ethnomedicine (Duke, 1985). The plant is a rich source of phytochemical such as alkaloids, terpenoids lignoids and cyclic peptides having a brand range of Bipotency (Devappa, 2010).

Alloxan has two distinct pathological effects namely selectively inhibiting glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensar of the beta cell, and causing a state of insulin-dependent diabetes through its ability to induce ROS formation resulting in the selective necrosis of the beta cells of the pancreas (Lenzen, 2008).

Materials and Methods
This work was carried out between the months of November, 2014 and June 2015 in the laboratories of the Department of Biochemistry of Kaduna State University, Kaduna – Nigeria.

Identification and Preparation of Plant Extracts
Fresh leaves and bark of *Jatropha curcas* were collected from the Nigerian Prison Barracks, Ungwan Rimi, Kaduna, Nigeria, were identified at the Herbarium of Biological Science Department, Ahmadu Bello University, Zaria, Nigeria and assigned a voucher Number 22373. The leaves and bark were freshly collected, washed and allowed to air-dry in shade at room temperature, after drying, they were pounded into fine powdery form, then soaked in 100% methanol for 48 hours, seined using a muslin cloth and rotary evaporated to remove excess methanol and then stored for further use.

Animal Material
Albino rats of both sexes were purchased from the Nigerian Institute for Trypanosomiasis Research (NITR), Ungwan Rimi, Kaduna, Nigeria, kept and maintained under laboratory conditions of temperature, humidity and light in the Department of Pharmacology Animal House of Kaduna State University, Kaduna Nigeria, the rats were also allowed free access to food and water *ad libitum*.

Induction of Diabetes Mellitus
Out of 21 Wistar albino rats obtained from Nigerian Institute for Trypanosomiasis Research (NITR), 12 were made diabetic by an intravenous injection of Alloxan 0.9gl 30ml of normal saline buffer. Diabetes was allowed to establish after 72 hours. The rats having blood glucose level of more than 250mglµ were randomly assigned into four (4) groups respectively.

Toxicity Test
Albino rats weighing 250-300g were used for the acute toxicity test experiments. The median lethal dose (LD_{50}) of *Jatropha curcas* leaves and bark methanolic extract were determined according to the method of Lorke (1983). Albino rats (not induced) fasted for 12hrs were randomly divided into 2 groups of 3 animals each, methanolic extract of both leaves and bark of *Jatropha curcas* 1000, 2000 and 4000mg were separately administered intra-peritoneally to the rats in each groups for the leaves and bark extract respectively. Each of the rats in the "normal control" group was treated with normal saline (2ml/kg) only. The Albino rats in both the test and controlled groups were then allowed free access to food and drinking water during which they were observed for 72hrs for signs of acute toxicity. The number of deaths (caused by the extracts) within this period of time was recorded. Log-dose plots were constructed for each of the plant extracts; from which the median lethal dose (LD_{50}) of the methanolic extracts was determined (Ojewole, 2003).

Experimental design
The rats were divided into seven (7) groups (n=3).
• Group 1 (used for toxicity test of Jatropha curcas leaves extract).
• Group 2 (used for the toxicity testy of *Jatropha curcas* bark extract).
• Group 3: CNI (used as normal control [not induced and not treated]).
• Group 4: CNT (used as negative control [induced but not treated]).
• Group 5: CSD (positive control [induced and treated with standard drug, Metcon 500-SR]).
• Group 6: JCB (rat with diabetes being treated with *Jatropha curcas* bark extract).
• Group 7: JCL (rat with diabetes being treated with *Jatropha curcas* leaves extract*).*

Determination of blood glucose
Both the normal control (normoglycemia) and alloxan-treated diabetic (hyperglycemia) rats were fasted for 12hrs but still allowed free access to drinking water. The blood glucose level of fasted alloxan-treated rats was

determined using a glucometer. At the end of the 12hrs, fasting period (taken as 0 time) blood glucose levels (initial glycaemia G_0) of the fasted normal (normoglycemia) and alloxan-treated diabetic rats were determined and noted. The normoglycemic animal was used as control for the hyperglycemic animals.

The plants extracts were administered intra-peritoneally following the administration of the plants extracts (leafs and bark), blood samples were collected from the (tails vain) of each rats (G_d) "blood glucose concentration" was determined by means of a glucometer and compactable blood glucose test strips. The percentage of glycemic variation was calculated as a function of days by applying the formula below:

$$\% \text{ glycemic change} = G_d - G_0 \times 100 / G_0$$

Where G_0 and G_d represent initial (zero time – 0 day) glycemic values before and glycemic values at 1,2,3,4,5,6, and 7 days after intra-peritoneally administrations of the test sample respectively.

The rats in the group treated with distilled water were used as controls for the experiment (Ojewole, 2003).

Data analysis

Blood glucose concentration data obtained from the blood samples of *Jatropha curcas* leafs and bark methanolic extracts as well as those obtained from distilled water-treated fasted "control" rats were expressed as mean (± SEM). The difference between the plant extracts, distilled water "control", standard drug "control" and diabetic "control" means were analyzed statistically by using "students T-test (Snedeeur and Cochrane, 1967) and One-way ANOVA using SPSS software version 16. Significant difference was accepted at ($p < 0.05$).

Results and Discussion

An *in-vivo* study of the antidiabetic potential of methanolic extract of the leaf and bark of *Jatropha curcas* was conducted on alloxan induced diabetic albino rats. The experiment was conducted within a period of 7 days.

The weight of the experimental animals was noted prior to diabetes induction with alloxan. Their weights were also noted during the period of the experiment. The blood glucose of the experimental animals was measured before and after induction; it was also noted throughout during the period of treatment with the plant extract so as to monitor the antidiabetic effect of both plant extracts. After, the 7 days period of the experiment, they were anaesthetized and dissected. The livers, lungs and kidneys were extracted, and taken to Zaria for histological analysis. The leaf and bark extracts of *Jatropha curcas* were also subjected to comparison to ascertain which among them had a higher antidiabetic property.

Data obtained were analyzed using student T-test and ANOVA. Significant difference was accepted at ($p < 0.05$).

Results

The medial lethal dose of this plant (*Jatropha curcas*) was investigated using a starting dose of 500mg/kg in the first animal in the first group to 1500mg/kg in the third animal in the same groups. This was done for both plant extracts (*Jatropha curcas leaves and bark*); the animals were then observed for 4 day during which on the second day mortality was recorded in one of the animals in group 2 which was treated with 1000mg/kg of the bark of the plant extracts; but, since there was no mortality recorded in the animals treated with a higher concentration of the plant extract, it was assumed that the animal did not die from toxicity of the plant extract rather, it might have died from injury sustained during the administration of the plant extract intra-peritoneally.

The initial body weight of all the animals in each group were measured and recorded before induction of diabetes with a diabetogenic chemical agent (alloxan). The weight range for all the groups was 102 – 290g (see table 1 below); there was a drastic drop in body weight of all the animals subjected to treatment with both plant extract and also in the 2 control groups (those induced with diabetes both not treated and those induced with diabetes and treated with standard drug [metcon 500 –SR]) (see table 1 below). But weight gain was observed in animals that were not induced neither treated with anything; which represent the normal control group.

Change in weight of animals before and after treatment.

Groups	N	Initial Weight (g) before induction	Final Weight (g) after treatment	*p*-value
CNI	3	215.85±62.86	215.93±64.86	>0.05
CNT	3	133.28±20.58	128.63±21.80	>0.05
CSD	3	122.03±27.68	116.49±28.92	>0.05
JCB	3	141.69±20.34	135.23±17.17	<0.05
JCL	3	179.41±21.15	169.91±25.34	<0.05

The values in the above table are given as mean ± S.D. the paired sample t-test was used to compare within the group and the level of significant is given as ($p < 0.05$). **CNI**-normal control, **CNT**-diabetic control, **CSD**-control treated with standard drug, **JCB**-treated with *Jatropha curcas* bark, **JLC**-treated with *Jatropha curcas* leaves.

From the above table, it was observed that there was no significant difference ($p < 0.05$) in the body

weight of all the animals which were in the control groups (CNI, CNT and CSD) but there was a significant difference (p>0.05) observed in the body weight of animals treated with both the plant extracts (JCB and JCL). Untreated diabetic rats had lower body weight when compared to the normal and treated groups. This is in line with some studies that reported significant weight reduction in untreated diabetic rats.

Blood samples were collected from all the rats on the first day before induction using a glucometer and noted; Blood samples were also collected and the blood glucose level measured and noted to establish whether the induction of diabetes was successful. It was observed that all the induced animals came up with diabetes mellitus (see table 2). The animals were then treated with the plant extracts (both that of the leaves and bark of the plant). Some were also treated with standard drug while others were not treated with anything; they were used as one of the controlled groups. The blood glucose levels of the various groups were measured throughout the period of the experiment (7days) and the values obtained were expressed in (mmol/l). Some changes were observed in their initial blood glucose level, after induction and during treatment (see table 2).

Effect of the methanolic leaves and bark extract of *Jatropha curcas* on the blood glucose level of alloxan induced diabetic albino rats.

GROUPS	Initial blood glucose (mmol/l) before induction	Blood glucose (mmol/l) after induction	Blood glucose (mmol/l) level during treatment		
			Day 1	Day 4	Day 7
CNI	5.3±0.7	5.1±0.4	4.6±1.4	5.1±0.3	5.5±0.7
CNT	5.2±0.4	17.0±5.5	24.7±13.3	26.0±2.5	29.5±3.9
CSD	5.4±0.5	23.7±5.5	8.4±1.1	5.7±0.6	4.4±0.3
JCB	5.0±1.4	13.8±5.5	7.9±2.3	4.7±0.6	3.4±0.2
JCL	6.3±0.7	19.9±8.3	12.4±6.2	5.3±0.6	4.4±0.6

The values in the above table are given as mean ± S.D. the paired sample t-test was used to compared within the groups, n=3: and the level of significant is given as (p<0.05). **CNI**-normal control, **CNT**-diabetic control, **CSD**-control treated with standard drug, **JCB**-treated with *Jatropha curcas* bark, **JLC**-treated with *Jatropha curcas* leaves.

Histological studies
Histological studies of the organs showed normal cellular architecture in organs of the controlled groups but pathological symptoms were observed in the lungs, kidney and liver of the animals that were treated with the plant extracts respectively.

Liver histology
From the plates, it was observed that there were structural changes to the central vein of the animals treated with the plant extracts as compared to those in the controlled group. The central vein of the liver of animals treated with the leaf extract showed more damage than that of those treated with the bark extract this could be due to a higher concentration of pharbol esters in the leaf of the plant which is the toxic component of the plant. There were also changes in the viable hepatocyte which showed shrinkage in those treated with the leaves extract while those treated with the bark extract showed enlargement as compared to those in the control group. There were no visible changes observed in the Kupffer cell of the animals treated with the bark extract.

Kidney histology
The control group and the animals in group treated with the leaf extract showed visible glomerulus and maintained their structural integrity while that of that of those treated with the bark extract showed signs of coagulative necrosis of the tubules in the cortico-medullary junction with cystic dilation and hemorrhage.

Lung histology
The bronchiole in all the animals treated with the plant extract (JCB and JCL) showed structural distortion as compared to that of animal in the controlled group, their alveoli cell also showed signs of necrosis as compared to that of the controlled group while the absent of the pulmonary vessel indicate their complete destruction in both groups of animals treated with the plant extract (JCB and JCL).

This study demonstrates hypoglycemic effect of the plant *Jatropha curcas* on the fasting blood glucose level of the rats (see table 2) and had a significant (P< 0.05) increase in blood glucose levels after induction with 150mg/kg of alloxan.

Conclusion
In view of the data obtained from this study, it shows that intraperitoneal administration of the leafs and bark of *Jatropha curcas* methanolic extracts (JCB and JCL) elicited antidiabetic properties by significantly lowering the blood glucose level in alloxan induced diabetic control groups which were not treated CNT. The hypoglycemic

and antidiabetic effect exhibited by the plant extract maybe as a result of the presence of high concentrations of alkaloids and flavonoids which had a concentration of 2.26mg and 3.83mg respectively (Nwamarah *et al.*, 2015). It was reported that medicinal plants with hypoglycemic and antidiabetic effect usually contains high concentration of alkaloids and flavonoids (Oladele *et al.*, 1995).

The treatment of diabetic patients with naturally derived agents has the advantage that it does not cause the significant side effects as do chemical agents such as sulfonylurea. One of the side effects with sulfonylurea is that it causes a decreased amount of insulin production by putting too great a strain on the insulin producing beta cells, treatment with herbal drugs has an effect of protecting beta cells and smoothing out fluctuations in glucose level (Venkateswaran and Pari, 2003).

REFERENCES CITED

American Diabetes Association, (2012): Diagnosis and Classification of Diabetes Mellitus. Diabetes Cure, 35(Suppl 1) 564-571.

Anita B.S., Okon Kan J.E., and Okon P.A. (2005): Hypoglycemic Effect of Aqueous Leaf Extracts of *Parsea Americana* mill on alloxan induced diabetic rats . Journal of Pharmacology 37:325-326.

Balaramwugan A., Mijamato B.J., Wang W., Inarne K., Tabata Y. (2003): Streptozotocin (STZ) used to induced diabetes in animal models. *Journal of Ethnopharmacology 26:102-103.*

Bliss M. (2000): The Discovery of Insulin University of Chicago Press, Chicago, U.S.A. pp 321-1418.

Choi S.B, (2014): Improvement of Insulin Resistance and Secretion by Water-extract of *Cardiceps milituris, Phellinus linteus* and *Pacerlamyce tenupes* in 90% paucreationized rats. *Journal of Biotechnology and Biochemistry. 68:2257-2267.*

Duke J.A. (1985): Medicinal Plans Science 229-1039.

Deveppa R.K., Makkar H.P.S and Becker K., (2010) Jatropha toxiocol Environ Health 13, 476.

Etuck, E.U., Mohammed, B.J. (2010), Evidence based analysis of Chemical Method of Induction of *Diabetes mellitus* in experimental animal, *Asian Journal Experiment Biological Science Vol. 1(2):331-336.*

Holemans K.I, and Vanassche F.A., (2003): Fetal Growth Restriction and Consequence for the Offspring in Animal models. ,Lenzen (2008): The Mechanisms of Alloxan and Streptozotocin-Induced Diabetes. Diabetologia 2008: 51(2): 216-226.

Larke D. (1983): A new approach to Practical Acute Toxicity Testing. Arch Toxicol 54(4):275-287.

Nwamarah J.U., Otitaju O., and Otitaju G.T.O (2015): Chemical Composition and Antidiabetic Properties of *Jatropha curcas* leaves extract on Alloxan induced diabetic Wister rats. *Afri Biotechnol Vol. 14(12) pp 1056-1066.*

Ojewole (2003): Laboratory Evaluation of the Hypoglycemic effect of *Anacardium occidentale* Linn (Anacardiaceae) Stem-bark extracts in rats. Methods Find Experiment Clin Pharmacol 2003 (25)(3): 199-204.

Oladele S.M., Ayo J.O., and Adaudi A.O. (1995): Medicinal and Physiological Properties of Flavonoid Cermiriva Derivatives and Anthraquines of Plant origin. *West Afri J. Pharmaocol Drug Res II: 134 – 144.*

Stener H., Oelzo O., Zehud G, Friesh E.R., (1970): Studies on Islet cell regeneration, hyperplasia and intramuscular Cellular interrelations in Long lasting Streptozotocin Diabetes rats Diabetologia 6:558-564.

Ven Kateswaran S. and Pari, L (2003): Effects of *Coccinca indica* leaf extract on plasma antioxidants in Streptozotocin induced experimental diabetes in rats Phytothera Research 17, 605-608.