Adetuyi et al., Biochem Pharmacol (Los Angel) 2015, 4:5
DOI: 10.4172/2167-0501.1000193

Biochemistry & Pharmacology: Open Access

Research Article Open Access

Anti-Hyperglycemic Potency of *Jatropha Gossypiifolia* in Alloxan Induced Diabetes

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Abstract

The aqueous extract of *Jatropha Gossypiifolia* were studied for their anti-hyperglycemic activity, fifteen animals were taken and they were divided into five groups. First group act as the control, while the remaining four groups were induced diabetics by administering alloxan (120 mg/kg). Second group induced with diabetes with insulin treatment , third group induced with diabetes without treatment, fourth was infected with diabetes treated with 12 hours intervals oral dose of ethanolic extract of leaves (120 mg/kgbody weight) and fifth group was infected with diabetes treated with 12 hours intervals oral dose of ethanolic extract of leaves (240 mg/kg body weight/day). From the experiments, It was discovered that the aqueous extract of *Jatropha Gossypiifolia* at dose of 120 mg/kg and 240 mg/kg for 7 days has effects in reducing hyperglycemia, it was discovered that the body weight increased, in line with one of the symptoms of diabetes; obesity. The test carried out on three different types of microbes shows that the aqueous extract inhibit the growth of the micro-organisms at different concentration. Staphylococcus aureus is a gram +ve bacteria, this can be seen in the low zone of inhibition seen in *Klebsiella pneumonia* (gram -ve) and also Escherichia coli is a gram – ve. It was also discovered that the ethanolic extracts of *Jatropha Gossypiifolia* have a phytotoxic effect; that is, they inhibit the growth of plants as compared to the control. This could be trace to the difference in the phytochemical components of the ethanolic extract, alkaloids, tannins, saponins and glucosides are discovered to be present in the ethanolic extract.

Keywords: Diabetes; *Jatropha Gossypiifolia*; Anti-hyperglycemic; Alloxan

Introduction

The incidence of each type of diabetes varies widely throughout the world. The vast majority of diabetes patients have type 2 DM. About 90% of all diabetic patients have type 2 DM. There are more than 125 million persons with diabetic in the world today. There is increase in current interest and demand for herbs as worldwide phenomenon, WHO currently encourages, contains curcin, a toxalbumin which is highly irritant and produces deleterious effects on blood. The latex is acrid and irritable to the skin. Curcain, a protease has been isolated from the latex of *Jatropha curcas* [1]. Some of the ethnomedical uses of *Jatropha curcas* have received support from the results of scientific investigations in recent times. For example, some compounds with antitumor activities were reportedly found in this plant.

Furthermore various solvent extracts of *Jatropha curcas* have an abortive effect [2]. Traditionally it is taken internally with ripe banana to treat dysentery in adults. The sap from twigs is considered styptic and is used for dressing wounds and ulcer. The bark rubbed with asafetida and buttermilk is reportedly taken internally in Konkan to relieve dyspepsia and diarrhea. A decoction of bark is used externally for treating rheumatism and leprosy. The decoction of root bark is used to rinse the mouth to relieve toothache and sore throat among the tribal inhabitants of

Southern Andhra Pradesh [3]. Some anecdotal reports are there, which reveals plant has been used in the indigenous system of medicine and literature in the treatment of various ailments. Its leaves have acrid taste, which is considered as an excretory product or secondary metabolites, may be of therapeutic use and have action on metabolism [4-6]. Since diabetes is considered as metabolic disorder so action on liver or pancreas can be postulated hence an attempt has been focused for its anti-diabetic activity.

In the present work, we investigated the anti-hyperglycemic potency of *jatropha gossypiifolia* in alloxan induced diabetes. Phytochemical screening was carried out on the plant extract, antimicrobial Sensitivity Assay was carried out. Also, the activities of serum and liver GGT, the activities of serum and liver BILIRUBIN, the activities of serum and liver ALP were evaluated in the treated rats. To the best of our knowledge, no data have been reported on the effects of these naturally occurring compounds on alloxan-induced diabetes., and, thus, the study was undertaken to fill the lacuna in this regard.

Animals

The albino rats (Rattus norvegicus) of both sexes, weighing from 120-180g were purchased from Ayo Ola Farms, Kwara State, Ilorin, Nigeria. They were kept under usual management conditions in conventional animal house of Biochemistry, Department of Chemical science, Joseph Ayo Babalola University, Ikeji-Arakeji, Ilesa, Nigeria. Rats were given standard laboratory diet and free access to water *ad libitum*.

Chemicals

Alloxan was purchased from Sigma Aldrich Chemicals Pvt , Ltd, Bangalore. All other chemicals and reagents used were of analytical grade.

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Received August 31, 2015; **Accepted** October 26, 2015; **Published** October 31, 2015

Citation: Adetuyi BO, Dairo JO, Didunyemi OM (2015) Anti-Hyperglycemic Potency of *Jatropha Gossypiifolia* in Alloxan Induced Diabetes. Biochem Pharmacol (Los Angel) 4: 193. doi:10.4172/2167-0501.1000193

Reagent kits for biochemical analysis (Infopia and Randox)

Glucose levels were measured using Fine Test strips and Fine Test Glucometer manufactured by Infopia Co. Ltd in Korea. Alkaline phosphates, were determined using commercially available reagent kits (Randox Laboratories Limited, UK). All other chemicals used were of analytical grade and obtained from BDH (London).

Plants Material

The plant *Jastropha Gossypiifolia* was selected for my study. The leaves of plant *Jastropha Gossypiifolia* (Family- Euphorbiaceae) was collected in the month of March, 2011 from Ikeji-Arakeji in Osun-State, Nigeria. The plant was taken to the Department of plant science Joseph Ayo Babalola University, Ikeji- Arakeji, Ilesa for identification.

Bacterial isolates

The microorganism for antimicrobial are *Escherichia coli, Klebsiella pneumonia and Staphylococcous aureus*

Extract preparation

The freshly collected leaves of *Jatropha Gossypiifolia* were first air dried for four days. It was blended into powder using an industrial blender. 50 g of crude fibre was extracted with ethanol by soaking in 500 ml of 98% ethanol for 72 hours. The sample was then filtered using whatman filter paper. The filtrate was evaporated into a syrup using a water bath at 70°C. The extract was stored in a refrigerator for use in subsequent experiment.

Phytochemical screening of *Jatropha Gossypiifolia*

Basic phytochemical screening analysis of ethanolic extract were carried out for the detection of Saponins, Alkaloids, Tannins, Anthraquinone, Ardenolides and phylobatannins.

Test for alkaloids: About 0.2 g of each extract was stirred in 5 ml of 1% hydrochloric acid in a steam bath. 1 ml of the filtrate was treated with few drops of drigen droff reagent. Turbidity or precipitation was taken as the preliminary evidence for the preliminary presence of alkaloids in the extract being evaluated Horbane, 1973.

Test for saponin: Saponin was detected by vigorously shaking 0.5 g of extract in 5 ml of distilled water in a test tube to observe a stable froth persistent on warming.

Test for tannins: About 0.2 g of each portion of plant extract was stirred with 5 ml of distilled water, filtered and ferric chloride (0.1%) reagent was added to the filtrate. A blue black green or blue green precipitate was taken as evidence for the presence of tannin Trease and Evan, 1978.

Test for phlobatannins: Deposition of red precipitate when an aqueous extract of the plant part boiled with 1% aqueous Hcl was taken as evidence for the presence of phylobatannins Trease and Evans, 1978.

Test for anthraquinone: About 0.2 g of the extract was shaken with 5 ml concentration benzene and 5 ml of 1% NH_3 solution was added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in ammoniacal (lower) phase indicate the presence of free anthraquinones.

Test for cardiac glucosides: The extract was dissolved in pyridine and few drops of 20% NaOH were added. A deep red colour, which faded to brownish yellow, indicate the presence of cardenlider.

Antimicrobial Sensitivity Assay

Media preparation

Nutrient Agar was prepared according to specification (Appendix 1) and then sterilized in autoclave at 121°C for 15 minutes. Agar plates were seeded with 0.5 ml of an overnight culture of each bacterial isolates: *E.coli*, *S. aureus, K. pneumonia.*

Extract were tested for inhibitory activity (invitro) against *Escherichia coli, Klebsiella pneumonia and Staphylococcous aureus* at different concentration of extract using the agar well diffusion (pop late technique method).

About 1 ml of 18 hours broth culture of the test organisms were introduced into a separated sterile petridish. Exactly 20 ml of sterile molten nutrient agar was poured into the petridish containing the test organisms. The agar was to set and holes were bored into the plates using sterile cork borer of 7 mm in diameter each. The wells bored on the plates were filled with the crude extract at different concentration of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml respectively. A control experiment was set up the same way, however instead of the extract, sterile water was introduced into the holes bored in each plate. The petri dishes were incubated upright at 37°C for 24 hours. The relative sensitivity of the organisms to the extract were indicated by clear zones of inhibition around the well which were observed, measured and recorded in millimeters.

Preparation of citrate buffer (0.1M at pH 4.5)

1.4705 g of sodium citrate was dissolve in the 50 ml of distilled water and was poured into a 100 ml volumetric flask on a magnetic stirrer, 1.125 g of citric acid is used to maintain the pH of the solution to 4.5.

Experimental design

Fifteen albino rats weighing between 50 – 160 g each were used for the experiment. They were divided into five groups with three rats per group. As shown in the (Table 1).

Experimental induction of diabetes

All animals were allowed to adapt to cages for 15 days, after which they were fasted overnight and 65 mg/kg of alloxan monohydrate freshly dissolved in normal citrate buffer was injected with intra-peritoneally. After alloxan treatment, all animals were given free access to food and water. Blood glucose levels were measured 5 days after alloxan injection. All treatments started 5 days after alloxan injection.

Serum and tissue preparation

At the end of the treatment period, rats in each group were starved overnight but had access to water *ad libitum,* weighed and sacrificed by cervical dislocation while under anesthesia, 2 ml blood was collected from each rat by cardiac puncture into plain tube [7,8]. The blood was allowed to stand for 5 minutes and then centrifuged at 3500 rpm (Beckman GS-6R, Germany) for 10 minutes at 4°C. Serum was obtained at the supernatant for measuring fasting blood sugar level and enzyme (ALP and GGT) levels [9,10]. Liver and kidney were quickly excised, rinsed in Isotonic Sterile Saline (ISS), bottled dry on a filter paper and weighed. Each tissue was then placed in a separate plastic vial containing ice-cold ISS and stored at -4°C until required for further analysis.

Groups of rats	No of rats	Description
A	3	Uninduced rats that were given 1 ml distitilled water daily.
B	3	Rats infected with diabetes with insulin treatment.
C	3	Rats infected with diabetes without treatment.
D	3	Rats infected with diabetes treated with 12 hours intervals oral dose of 120mg/kg b.w. aqueous extract of Jatropha Gossypiifolia.
E	3	Rats infected with diabetes treated with 12 hours intervals oral dose of 240 mg/kg b.w. aqueous extract of Jatropha Gossypiifolia.

Table 1: Rats grouping.

Preparation of tissue homogenate

1 g of each of liver and kidney was cut out chopped into small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenate 5 ml of Isotonic Sterile Saline and centrifuge at 3500 rpm for 10 minutes and stored at -4°C until further analysis was carried out.

Biochemical assays

Serum glucose concentration was determined by means of Bayer Elite. Alkaline Phosphatase (ALP), Total Billirubin and GGT activities were determined using commercially available enzymatic test kits (Randox Laboratories Ltd, San Francisco, USA) method following the manufacturer's instructions.

Results

Phytochemical screening

The table below shows the result obtained for the phytochemical screening of the ethanolic extract Jatropha Gossypiifolia. The ethanolic extract revealed the presence of alkaloids, saponins, tannins and glycosides (Table 2).

Antimicrobial sensitivity assay

The table below shows the inhibition zone (mm) for the antimicrobial activity of ethanolic extract *Jatropha Gossypiifolia* at different concentration against bacterial isolates (Table 3) (Figures 1-3).

Result of body weight and blood glucose level

The Result of body weight and blood glucose level are shown in (Table 4).

Biochemical Assay

This shows the result of effect of ethanolic extract of *Jatropha Gossypiifolia* leaf on the activities of serum and organs in GGT, Bilirubin and ALP of serum and organ damage in rats. Each value is a mean of '3 determination ± S.D (Figure 4-6) Graph of ALP activity against Treatment Groups (Figure 5).

Discussions

In light of the result, our study indicate that ethanolic extract of *Jatropha Gossypiifolia* have good antidiabetic activity but there was a paralysis in one leg and lesion on the side of the paralysed legs, and some behavioural sign like loss of appetite and tiredness was observed.

Diabetes mellitus arises from the irreversible destruction of the pancreatic beta cells causing degranulation and reduction of insulin secretion. The renewal of beta cells in diabetes have been studied in several animals model [11]. The present study demonstrated that the 50% ethanolic extract of *Jatropha Gossypiifolia* had an antihyperglycemic effect in the alloxan induced diabetic rats when

Figure 1: Inhibition zone of the ethanolic extract on *Escherichia coli*.

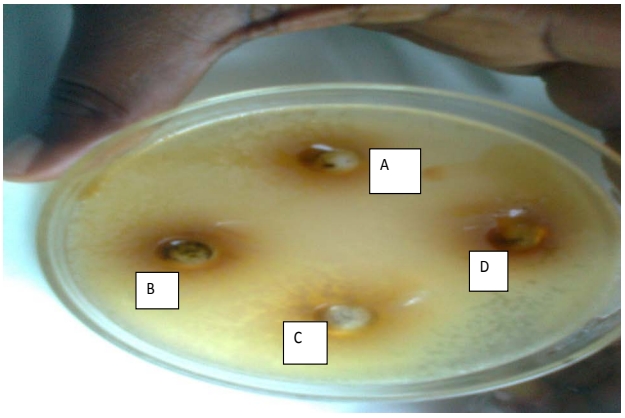


Figure 2: Inhibition zone of the ethanolic extract on *Klebsiella pneumonia*.

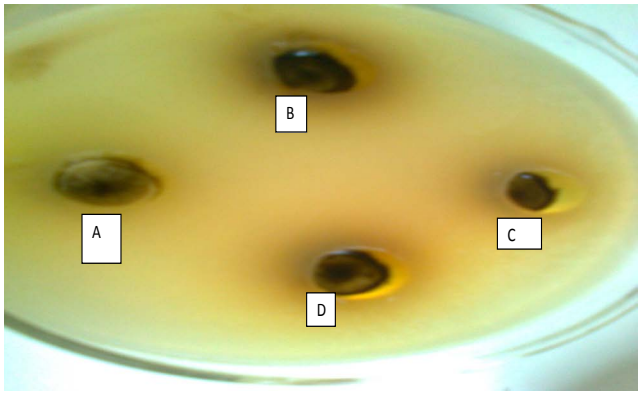
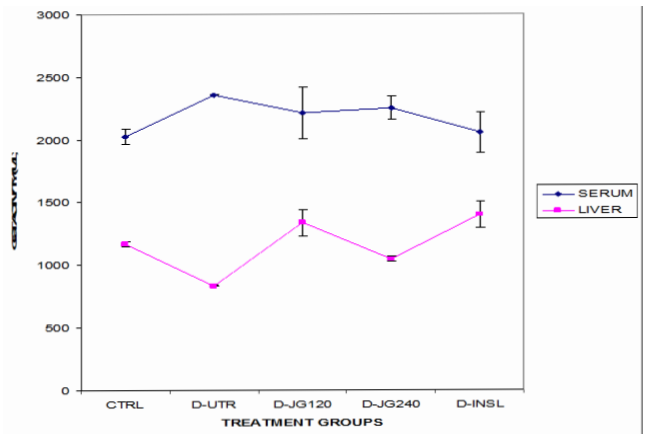
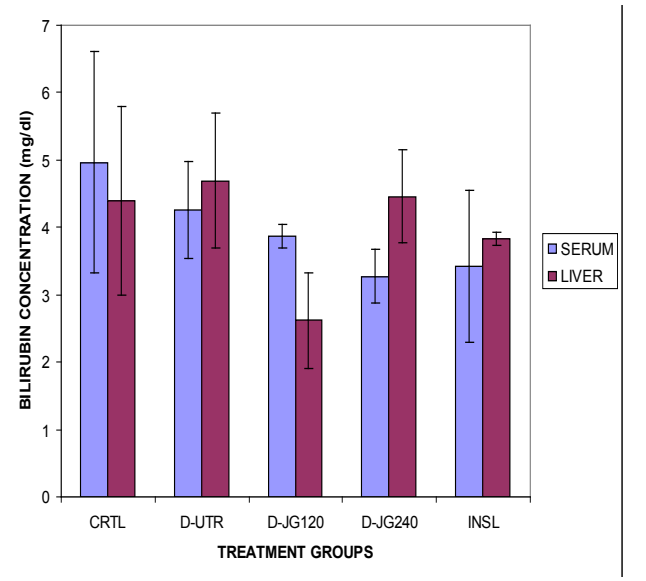


Figure 3: Inhibition zone of the ethanolic extract on *Staphylococcus aureus*.



Note: CTRL - CONTROL RATS
D-UTR – DIABETES RATS WITHOUT TREATMENT
D-JG120- DIABETES RATS TREATED WITH *JATROPHA GOSSYPIIFOLIA* CONCENTRATION OF 12O mg/kg
D-JG240- DIABETES RATS REATED WITH *JATROPHA GOSSYPIIFOLIA* CONCENTRATION OF 240 mg/kg
D-INSL – DIABETES RATS TREATED WITH INSULIN

Figure 4: Effect of ethanolic extract of *Jatropha Gossypiifolia* leaf on the activities of serum and liver GGT of serum and liver damage in rats. Each value is a mean of '3 determination ±S.D.



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D-INSL – DIABETES RATS TREATED WITH INSULIN

Figure 5: Effect of ethanolic extract of *Jatropha Gossypiifolia* leaf on the activities of serum and liver BILIRUBIN of serum and liver damage in rats. Each value is a mean of '3 determination ±S.D.

administered orally. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory process, which in turns leads to accumulation of lipids such as cholesterol and triglyceride in diabetic patients. The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots [12]. In present study, the 50% ethanolic extract of leaves of *J. curcas* decrease the cholesterol and triglyceride levels in the significant manner.

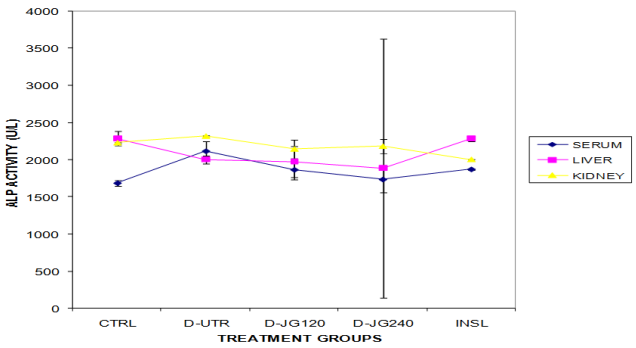
From the experiments conducted in the albino rats. It was found that the aqueous extract of *Jatropha Gossypiifolia* at dose of 120 mg/kg and 240 mg/kg for 7 days has effects in reducing hyperglycemia, it was seen that the body weight increased, in line with one of the symptoms of diabetes; obesity [13].

The test carried out on three different types of microbes shows that the aqueous extract inhibit the growth of the micro-organisms at different concentration. *Staphylococcus aureus* is a gram +ve bacteria, this can be seen in the low zone of inhibition seen in *Klebsiella pneumonia* (gram -ve) and also *Escherichia coli* is a gram – ve.

From (Table 2), it was discovered that the ethanolic extracts of *Jatropha Gossypiifolia* have a phytotoxic effect; that is, they inhibit the growth of plants as compared to the control. This could be trace to the difference in the phytochemical components of the ethanolic extract, as observed in this research project, alkaloids, tannins, saponins and glucosides are discovered to be present in the ethanolic extract.

Conclusion

The ethanolic extract of *Jatropha Gossypifolia* effectively reduced the alloxan-induced changes in blood glucose level. The current study provides some useful insight into the antihyperglycemic potency of *Jatropha curcas* leaves in alloxan induced diabetes. However, we



Note:
CTRL - CONTROL RATS
D-UTR – DIABETES RATS WITHOUT TREATMENT
D-JG120- DIABETES RATS TREATED WITH *JATROPHA GOSSYPIIFOLIA* CONCENTRATION OF 12O mg/kg
D-JG240- DIABETES RATS TREATED WITH *JATROPHA GOSSYPIIFOLIA* CONCENTRATION OF 240 mg/kg
D-INSL – DIABETES RATS TREATED WITH INSULIN

Figure 6: Effect of ethanolic extract of *Jatropha Gossypiifolia* leaf on the activities of serum, kidney and liver ALP of serum and liver and kidney damage in rats. Each value is a mean of '3 determination ± S.D.

s/no	Phytochemical component	Jatropha Gossypiifolia ethanolic extract
1	Alkaloids	Positive
2	Saponins	Positive
3	Tannins	Positive
4	phlobatannins	Negative
5	Anthraquinone	Negative
6	Glycosides	Positive

Table 2: Result of phytochemical screening.

Test organism	Different concentration			
	25 mg/ml (mm)	50 mg/ml (mm)	75 mg/ml (mm)	100mg/ml (mm)
Escherichia coli	12	8	13	11
Klebsiella pneumonia	14	10	12	15
Staphylicoccous aureus	10	12	13	14

Table 3: Result for Inhibition zone of antimicrobial sensitivity assay.

GROUP	Body weight (g)			Blood Glucose (mg/dl)		
Groups	Final	Initial	% Change	Final	Initial	% Change
Group A(Control)	95.27 ± 16	93.13 ± 9.2	2.14	100.2 ± 0.5	92.1 ± 7.5	8.79
Group B(UTR-D))	130.0 ± 4.6	154.8 ± 18	24.8	193.12 ± 4.2	158.32 ± 4.9	21.98
Group C(DJG120)	87.167 ± 2.9	95.53 ± 1.2	8.36	111.5 ± 20	288.5 ± 6.9	61.35
Group D(DJG240)	124.167 ± 5.0	137.3 ± 19	13.33	178 ± 3.3	248 ± 32	28.22
Group E(Insulin)	196.4 ± 5.3	146.17 ± 2.5	50.23	118 ± 14	121.67 ± 22	3.02

The results are the mean of 3 determinations ± S.D

Table 4: Weight and blood glucose in experimental and control rats before and after treatment with aqueous extract of ethanolic *Jatropha gossypifolia leaves.*

suggest that further work should be carried out at molecular level to find out the absolute mechanism of action of plant *Jatropha Gossypifoli* in experimental diabetes.

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