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Research Article

# Acute toxicity and antihyperglycaemic effect of ethanol stem-bark extract of *Annona senegalensis Pers. (Annonaceae)* on alloxan induced diabetic rats

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**Background:** Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries. Many oral hypoglycaemic agents available for the treatment have significant side effects and sometimes are found to be ineffective in chronic diabetic patients.

**Objective**: To study the acute toxicity and effect of the ethanol stem-bark extract of *Annona senegalensis* on blood glucose in alloxan-induced diabetic rats.

**Material and Methods**: Hypoglycaemic effect of the extract was studied in normal rats using oral glucose tolerance test and in alloxan-induced diabetic rats. Effects of 100, 200 and 300 mg/kg, *i.p* of ethanol stem-bark extract of *Annona senegalensis*, and glibenclamide (5 mg/kg, i.p) were studied on blood glucose.

**Results**: The median lethal dose of the extract was found to be 1131.4 mg/kg by *intraperitoneal* route. In the normal rats (OGTT), from basal to 0 min, the extract did not show significant reduction in the fasting serum glucose level. However, the extract at 300 mg/kg and glibenclamide (5 mg/kg) significantly ($p \leq 0.05$) prevented glucose induced hyperglycemia at 30 to 90 min as compared to normal control. In the alloxan-induced diabetic rats, 100, 200 and 300 mg/kg of the extract produced significant ($p \leq 0.05$) reduction in blood glucose levels. Maximum effect was observed with the 300 mg/kg dose of stem-bark of *Annona senegalensis*.

**Conclusion**: The results showed that the ethanol extract of the stem bark of the plant possessed antihyperglycaemic activity.

**Key Words**: Hypoglacaemic effect, *Annona senegalensis*, diabetes mellitus, glibenclamide.

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## 1. Introduction

Diabetes is a chronic disorder in metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance. (Barar, 2000). It may also be defined as a disease where the body either produces little insulin or ceases to produce insulin, or becomes progressively resistant to its action (Ran and Ramanu, 2002). Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries (Erasto et al, 2005). It is ranked seventh among the leading causes of death, and third when all its fatal complications are taken into account, because of its high prevalence, morbidity and mortality (Trivedi et al, 2004). The

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number of individuals suffering from diabetes in Nigeria for instance is expected to increase from 3, 746, 5000 in 2014 (IDF 2014) to 4, 835, 000 by 2030 (WHO, 2010). Many oral hypoglycaemic agents, such as biguanides and sulfonylureas are available along with insulin for the treatment of diabetes mellitus, but they have significant side effects and sometimes they are found to be ineffective in chronic diabetic patients (Defronzo and Goodman 1995; Nathan et al*,* 2006). Thus, there is an increasing demand of natural and synthetic products with high antidiabetic potential and lesser side effects. The research conducted over the last several decades has shown that plant and plant-based therapies have high potential to treat and control diabetes and its complications (Singh et al*,* 2008). Diabetes has been treated orally with several medicinal plants or their extracts, based on folklore medicine. Therefore, search for safe and more effective agents has continued to be an important area of active research.

*Annona senegalensis* Pers. (*Annonaceae*) commonly known as "wild custard apple" is widely grown in Nigeria where it is commonly known as "Gwandar daaji" in Hausa, and "abo emeaso" in Yoruba (Alawa et al*,* 2003; Sulaiman et al*,* 2008). The plant grown as a shrub or small tree, has aromatic flowers which are used to flavour food. The ripe fruit is yellow in colours and has a sweet edible jelly with pleasant odour.

The biological activities of the plant extract reported by many researches justified the use of the plant in traditional medicine. For instance, the plant extracts were found to possess antimalarial, antidiarrhoea, antibacterial and anthelmintic properties (Alawa et al*,* 2003; Ajaiyeoba et al*,* 2006). The plant decoction is used in the treatment of sleeping sickness in northern Nigeria (Igwe and Onabanjo, 1989) and in folkloric treatment of cancer (Graham et al*,* 2000; Abubakar et al*,* 2007). It is used in chest pain, coughs, anaemia, urinary tract infections (Muanza et al*,* 1994) as well as intestinal troubles and stomach ache (Dalziel, 1955), diarrhea, body stool, dysentry (Muanza et al*,* 1994; Ekpendu et al*,* 1998) and veneral disease (Durodola, 1975). The plant has also been shown to be beneficial in the treatment of snake bite (Adzu et al*,* 2005).

In the light of the above data, the objectives of the present study were to evaluate (1) the acute toxicity of the stem bark extracts of *A. senegalensis* and (2) effect the extracts on blood glucose in normal as well as alloxan-induced diabetic rats.

## 2. Materials and Methods

### 2.1 Plant Collection

The stem bark of the plant, *Annona senegalensis* was collected Yako in Kiru Local Government Area of Kano State. The plant specimen was identified by Mal. Baba Ali Garko and authenticated by Prof. Bala Sidi of Biological Sciences Department, Bayero University, Kano where a voucher number (**BUKHAN0029**) was assigned for reference.

### 2.2 Chemicals

All chemicals and drugs were obtained commercially and were of analytical grade. Alloxan monohydrate (Sigma Aldrich, St. Louis, MO, USA) Glibenclamide (NAFDAC Reg. No 04-1601).

### 2.3 Experimental Animals

Adult Wistar rats (200-250 g) of either sex were obtained from Department of Pharmacology, Faculty of Medicine, Bayero University, Kano and used for the study. The animals were kept in standard cages in the departmental animal house for two weeks to acclimatize. Animals were fed with commercial feed (vital feed) and water was given ad libitum. Animals described as fasted were deprived of food for 18 h but had free access to water.

### 2.4 Extraction

The plant material was dried under shade and then pulverized into powder. The powder was percolated with 96% v\v ethanol for two weeks. The ethanol extract was filtered and concentrated using rotary evaporator at 40°C. The crude extract obtained was kept in deep freezer until use.

### 2.5 Phytochemical screening

The preliminary phytochemical screening of the crude ethanol stem bark extract of *A. senegalensis* was carried out in order to ascertain the presence of alkaloids, saponins, steroids, flavonoids and reducing sugar using protocols described in Trease and Evans (1983).

### 2.6 Acute toxicity study

The median lethal dose ($LD_{50}$) of the ethanol stem bark extract of *A. senegalensis* was determined by method of Lorke (1983). In the initial phase 9 rats were divided into 3 groups of 3 rats each and treated with the extract at doses of 10, 100 and 1000 mg/kg body weight *intraperitoneally* (*i.p*) and observed for 24 hours for signs of toxicity or death. In the second phase, another 4 rats were divided into 4 groups of 1 rat each and were administered with the extract at the doses of 200, 400, 800 and 1600 mg/kg body weight respectively (*i.p*). The animals were all kept under same conditions and mortality was recorded in each group within 24 h.

### 2.7 Oral Glucose Tolerance Test (OGTT)

Oral Glucose Tolerance Test (OGTT) in normal rats was performed by dividing fasted rats into groups of five. Group I served as normal control and received distilled water. Group 2, 3 and 4 received the 100 mg/kg, 200 mg/kg and 300 mg/kg of the extract as an aqueous suspension respectively. Group 5 received the reference drug Glibenclamide 5 mg/kg. The serum glucose level for all the groups were estimated prior to the administration of the extract and reference drug and were considered as basal readings. Immediately after blood withdrawal, all the experimental groups were administered with respective doses of the extract and reference drug.

After 30 minutes of administrations, the serum glucose level of all groups was recorded to evaluate the hypoglycaemic effect of extract and reference drug in normal rats and these readings were considered as the readings at 0 min. Glucose (2 g/kg) was loaded in all the

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groups at 0 min and blood samples were collected from the rat tail vein at 30, 60, 90 and 120 minutes after glucose loading. Blood glucose levels were measured using ACCU-CHEK (active) glucometer.

### 2.8 Alloxan Induced hyperglycaemia

The rats were fasted for 12 hours before the commencement of the experiment, but were allowed to take water *ad libitum*. Their fasting blood glucose was taken before being treated with alloxan. Diabetes was then induced by a single *i.p.* of alloxan monohydrate at a dose of 150 mg/kg body weight to each rat. The rats were kept for the next 24h on 5% glucose solution to prevent hypoglycaemia. The diabetic state was assessed by measuring the blood glucose levels after 72h. The rats with blood glucose levels above 199 mg/dl were considered diabetic and included in the study. Blood samples were obtained from the tail end of each rat. The blood glucose estimations were made using ACCU-CHEK (active) glucometer, Johnson Company, USA.

Rats with blood glucose level between 200 and 400 mg/dl were selected and divided into five groups each containing five rats as follows:

Group 1, served as untreated diabetic control.

Group 2, served as diabetic-treated with 100 mg/kg extract.

Group 3, served as diabetic-treated with 200 mg/kg extract.

Group 4, served as diabetic-treated with 300 mg/kg extract.

Group 5, served as diabetic-treated with 5 mg/kg glibenclamide (standard control).

Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 0, 2, 4, 8, 16 and 24 hours using ACCU-CHEK (active) glucometer, Johnson Company, USA instrument.

### 2.9 Statistical analysis

The data were expressed as mean ± SEM. Statistical analysis was performed using Microsoft Excel 2010 Analysis ToolPak. The effect of the extract was evaluated for each group in the alloxan–induced diabetic model by calculating the percentage glycaemic change using following formula:

$$\%\ \text{Glycaemic change} = \frac{(G_i - G_t)}{G_i} \times 100$$

Where:

Gi = the value of initial glucose concentration (0 min in OGTT and alloxan-induced diabetes model)

Gt = glucose concentration at different intervals.

Positive (+) indicates decrease and negative (-) signifies increase. The treatments and control groups were compared using paired Student's t-test, values of $p \leq 0.05$ were considered statistically significant.

## 3. Results

Phytochemical investigation of the freshly prepared extract revealed the presence of alkaloid, saponins and flavonoids as secondary metabolites. Steroids and reducing sugars were absent.

The *intraperitoneal* median lethal dose ($LD_{50}$) of the ethanol stem back extract of the *A. senegalensis* in rats was calculated to be 1131.4 mg/kg body weight using Lorke (1983) Method (**Table 1**). Other signs of toxicity were observed from 600 mg/kg and include decrease in locomotor activity, piloerection and sensitivity to touch as well as feed intake. The severity of the signs increased with increasing dose.

**Table 1**: Mortality of Ethanol Stem Bark Extract of *Anona senegalensis* in Wistar Rats

| Initial Phase | | Second Phase | |
|---|---|---|---|
| **Dose (mg/kg)** | **Mortality** | **Dose (mg/kg)** | **Mortality** |
| 10 | 0/3 | 200 | 0/1 |
| 100 | 0/3 | 400 | 0/1 |
| 1000 | 2/3 | 800 | 0/1 |
| | | 1600 | 1/1 |

Mortality is expressed as number of animal which died/number of animal(s) used.

### Oral glucose tolerance test

The serum glucose level, after oral administration of glucose in normal control and treated rats are given in **Table 2**. Neither the reference drug (glibenclamide) nor the extract showed any hypoglycaemic effect in normal rats as evident by basal to 0 min. readings. Thirty (30) minutes after glucose administration the blood glucose level increased rapidly from the fasting value and peak 60 min, then subsequently decreased. As expected, glibenclamide (5 mg/kg) was observed to prevented glucose induced hyperglycemia significantly at 30 to 90 min ($p \leq 0.05$) as compare to normal control. The extract (200 and 300 mg/kg) at 60 to 90 min also significantly ($p \leq 0.05$) prevented the hyperglycaemia. Maximum glucose tolerance in *Annona senegalensis* extracts was observed with 300 mg/kg in 90 minutes compared with the normal control.

### Alloxan Induced hyperglycaemia

Evaluating the effect of the extract on blood glucose levels of alloxan-induced diabetic rats showed that, the extract improved the percentage glycaemic change by

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lowering the blood glucose level (10-19%) in dose dependent manner within the first 2 hours of the extract administration. The single dose (300 mg/kg) of the ethanol extract of *Annona senegalensis* showed significant reduction (p≤0.05) at two hours that was comparable to that the reference drug. Further significant (P<0.005) decrease (28-33%) was observed after 4 hours of the extract administration at 200 and 300 mg/kg when compared to the control. The significant decrease was sustained up to 16 hours post administration by 300 mg/kg of the extract. Though 100 and 200 mg/kg body weight of the extract also sustained the blood glucose levels lowering 8 to 16 hours after administration, the percentage glycaemic change was not statistically significant. Maximum blood glucose level lowering effect (40%) was observed with 300 mg/kg 8 hours after administration of the extract when compared to diabetic control (**Table 3**). No significant improvements in percentage glycaemic change were recorded with all the three doses of the extract after the 24th hour.

As expected, negative percentage glycaemic change (increase in blood glucose level) was observed from 4 up to 24th hour after administration of normal saline in the control group. In contrast, the reference drug (5 mg/kg glibenclamide) was found to non-significantly lower the blood glucose level at 8th hour after administration and significantly improved the percentage glycaemic change at 4th hour (p< 0.05) and between 16 and 24th hour (p< 0.005) when compared to diabetic control rats.

**Table 2**: Effect of the Ethanol Stem Bark Extract of *Anona senegalensis* on Serum Glucose Levels on Glucose Loaded Normal Rats (OGTT study)

| **Dose** | **Serum glucose level (mg/dl)** | | | | | |
|---|---|---|---|---|---|---|
| | **Basal** | **0 min** | **30 min** | **60 min** | **90 min** | **120 min** |
| N/S 10ml/kg | 93.17 ± 0.83 | 91.83 ± 1.01 | 133.33 ± 1.91 | 157.67 ± 2.30 | 125.33 ± 1.38 | 94.20 ± 1.16 |
| Extract (100mg/kg) | 93.50 ± 1.43 | 91.67 ± 0.80 ns | 132.67 ± 0.99 ns | 152.33 ± 1.82 ns | 124.83 ± 1.11 ns | 94.67 ± 0.84 ns |
| Extract (200mg/kg) | 91.50 ± 1.59 | 90.83 ± 1.10 ns | 131.67 ± 1.13 ns | 147.67 ± 0.67 a | 119.83 ± 1.45 a | 89.67 ± 2.91 ns |
| Extract (300mg/kg) | 92.83 ± 1.28 | 92.17 ± 1.30 ns | 130.00 ± 0.97 ns | 145.50 ± 1.41 a | 118.17 ± 1.66 b | 89.17 ± 2.98 ns |
| Glibenclamide (5mg/kg) | 91.73 ± 1.39 | 91.17 ± 1.54 ns | 127.83 ± 1.49 a | 143.83 ± 1.56 b | 118.00 ± 1.65 b | 91.67 ± 1.54 ns |

**Key**: ns = Not significant; a =P<0.05; b = P<0.005 compared to control (N/S) using One way Anova followed by Dunnett's post hoc test

**Table 3:** Effects of Ethanol Stem Bark Extract of *Annona senegalensis* on Induced Diabetic Wistar Albino Rats

| Group | **% Glycemic Change (mean ± SEM)** | | | | | |
|---|---|---|---|---|---|---|
| | **0 hr** | **2 hr** | **4 hr** | **8 hr** | **16 hr** | **24 hr** |
| N/S 10ml/kg | 0 ± 0.00 | 0.31 ± 3.95 | -7.41 ± 2.00 | - 11.23 ± 6.24 | - 20.35 ± 9.48 | -31.67 ± 8.66 |
| Extract (100mg/kg) | 0 ± 0.00 | 10.66 ± 9.51 ns | 16.60 ± 8.51 a | 6.36 ± 10.29 ns | 4.28 ± 14.98 ns | 2.48 ± 3.29 ns |
| Extract (200mg/kg) | 0 ± 0.00 | 10.97 ± 6.87 ns | 27.85 ± 5.35 b | 21.75 ± 24.74 ns | 3.61 ± 24.51 ns | 3.53 ± 17.58 ns |
| Extract (300mg/kg) | 0 ± 0.00 | 18.99 ± 7.46 a | 24.68 ± 5.20 b | 40.21 ± 10.27 a | 32.73 ± 9.72 b | 8.09 ± 5.07 ns |
| Glibenclamide (5mg/kg) | 0 ± 0.00 | 17.26 ± 1.24 a | 20.68 ± 17.98 a | 22.87 ± 10.39 ns | 28.15 ± 2.86 b | 19.17 ± 0.83 b |

**Key**: ns = Not significant; a =P<0.05; b = P<0.005 compared to control (N/S) using One way Anova followed by Dunnett's post hoc test
Positive (+) percentage glycaemic change indicates decrease and negative (-) percentage glycaemic change signifies increase. Calculated as described in text

## 4.0 Discussion

Phytochemical investigation of the freshly prepared extract revealed the presence of alkaloid, saponins and flavonoids as secondary metabolites. Similar result was obtained on root bark extract of the plant by Okoye et al, (2012). Many of these compounds have been shown to produce potent hypoglycaemic, antihyperglycaemic, and glucose suppressive activities (Tanko et al, 2008). These effects might be achieved by facilitating insulin release from beta pancreatic cells, inhibiting glucose absorption in gut, stimulating glycogenesis in liver and/or increasing glucose utilization by the body.

In acute toxicity study, decrease in locomotor activity and sensitivity to touch as well as decreased feed intake

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were observed 8–12 hours after administration of the extract in the second phase. The median lethal dose ($LD_{50}$) of the ethanol stem back extract of the *A. senegalensis* in rats was calculated to be 1131.4 mg/kg body weight. The $LD_{50}$ obtained suggests that the ethanol stem bark extract of *A. senegalensis* may be less toxic with some risk of acute intoxication at high doses in rats.

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose insulin is secreted (Edem, 2009). Although the precise mechanism of alloxan-induced diabetes remains unclear, there is increasing evidence that alloxan preferentially accumulate in pancreatic β-cells via the GLUT2 glucose transporter. In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the β-cells, which have a particularly low antioxidative defence capacity, and the ensuing an insufficient release of insulin (Lenzen 2008) from the β-cells of the islets of Langerhans. An insufficient release of insulin, leads to high blood glucose level or hyperglycemia (Grover et al*,* 2002). Insulin deficiency leads to various metabolic alterations in the animals, viz. increased blood glucose, increased cholesterol and transaminases (Shanmugasundaram et al*,* 1983). The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (Jayasri et al*,* 2008).

In the present study the antihyperglycaemic activity of ethanol stem bark extract of *Annona senegalensis* was evaluated in normal (OGTT) and in alloxan induced diabetic rats, respectively. Notable antihyperglycaemic activity was observed with the *Annona senegalensis* crude extracts both in the normal (OGTT) and in alloxan induced diabetic rats. *Annona senegalensis*, being an antioxidant (Ajboye et al*,* 2010) may prevent damage to the pancreatic islet cell induced by the cytotoxic oxygen radicals (Bhattacharya, 1995). Another reason for the plasma glucose lowering action may be due to the decreased gluconeogenesis, which appears to be related to the antioxidant properties of the plant extract (Ortiz-Andrade et al*,* 2007). The antihyperglycaemic effect may also be due to the presence of insulin-like substance found in various plants (Gray and Flatt 1999).

The reference drug (5 mg/kg glibenclamide) was found to significantly improve the percentage glycaemic change between 16 and 24$^{th}$ hour, which could be explained by longer half-life of glibenclamide (2-24h).

## 5.0 Conclusion

The findings of this study indicate the presence of various phytochemicals in the plant extracts, which may be responsible for the pharmacological activity. The extract significantly decreased the blood glucose levels in dose dependent manner. The extract at 300 mg/kg appear to have comparative effect with glibenclamide 5 mg/kg. Further studies is recommended that may lead to possible isolation of active molecule and evaluating its safety profile.

## Conflict of Interest declaration

The authors declare no conflict of interest.

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