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Anti-hyperglycemic Effect of *Khaya senegalensis* Stem Bark Aqueous Extract in Wistar Rats

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Research Article

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ABSTRACT

Aim: To investigate anti-hyperglycemic effect of aqueous extract of *Khaya senegalensis* stem bark (KSE) in alloxan-diabetic Wistar rats.
Methodology: Thirty rats were randomly divided into six groups of 5 animals each. Group I (non-diabetic control) was given distilled water orally. Animals in the remaining five groups were treated with a single dose of alloxan (120mg/kg body weight, i.p) to induce diabetes mellitus. This resulted in significant increase in the fasting blood glucose level of the rats. Group I (non-diabetic control) and group II (hyperglycemic control) then received distilled water orally for 14 days. Group III, IV and V were treated orally with daily doses of 50, 100 and 150 mg/kg body weight of KSE respectively for 14 days. Group VI was given glibenclamide (5mg/kg, p.o) for the same period. Fasting blood glucose was determined by oxidative method in all the groups on day 0 (before treatment), day 7 and day 14. Oral glucose tolerance test and erythrocyte malondialdehyde (MDA) concentration were estimated after the two week treatment. Body weights of the animals were also measured on day 0, day 7 and day 14.
Results: Treatment with KSE and glibenclamide caused significant ($p<0.05$) and dose-dependent changes compared to the untreated animals with respect to body weight, blood glucose level and erythrocyte malondialdehyde (MDA) concentration. The anti-hyperglycemic effect of KSE was comparable to that of the standard drug, glibenclamide.
Conclusion: The study showed that aqueous extract of *Khaya senegalensis* stem bark possesses anti-hyperglycemic activity.

Keywords: Diabetes; lipoperoxidation; erythrocyte; alloxan; glibenclamide.

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1. INTRODUCTION

Diabetes mellitus is a major health problem around the world and its prevalence is increasing at alarming rate (Pavana et al., 2008). According to the World Health Organization, an estimated 3% of the world's population have diabetes and this is expected to double by the year 2025 (Andrade-Cetto and Heinrich, 2005). The reasons for this projected increase include rapid urbanization and westernization and the associated lifestyle changes. Genetic predisposition is also a factor contributing to this menace (Wild et al., 2004). Key features in the pathogenesis of diabetes mellitus are decreased ability of insulin to stimulate glucose uptake in peripheral tissues, insulin resistance and β-cell failure (White et al., 2003). Exercise, diet restriction and modern drugs including insulin and oral hypoglycemic drugs such as biguanides and sulfonylureas have been employed in the management of diabetes mellitus. However, management of diabetes with drugs that have minimal side effects is still a challenge in the medical field. This has led to a relentless search for improved anti-diabetic drugs. This search includes plants that are used traditionally for the treatment of diabetes (Assubaie and El-Garawany, 2004). From time immemorial, medicinal plants have been used in virtually all culture as a source of medicine and many of the modern drugs originated from these plants (Falodun, 2010). It has been estimated that about 80 – 85% of the population in developing nations rely on traditional system of medicine for their primary health care needs and a major part of traditional therapy involves the use of herbal drugs (Ignacimuthu et al., 2006). Before the discovery of insulin by Bantin and Best in 1922, the treatment options for diabetes are basically those of traditional practice (Ribnicky et al., 2006). Among medicinal plants used traditionally for the treatment of diabetes are *Ajuga remota* (Abebe et al., 2003), *Momordica charantia* (Kolawole et al., 2011) and *Khaya senegalensis* (Chattopadhyay, 1996). *Khaya senegalensis* (Desr.) belongs to the family *Meliaceae* (mahogany family). It is a popular medicinal plant widely used to treat various kinds of diseases in Nigeria and other West Africa countries. In this study, we investigated the anti-hyperglycemic effect of aqueous extract of *Khaya senegalensis* stem bark in diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

Stem bark of *Khaya senegalensis* was collected from Igbona area, Osogbo, Nigeria during the month of January. It was identified and authenticated by a taxonomist in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, and voucher specimen was deposited in the herbarium of the Department. The study was conducted between January and April, 2011.

2.1.1 Extraction procedure

The dry stem bark of *K. senegalensis* was cut into small pieces and pulverized into fine powder using an electric grinding machine. 300g of the sample was soaked in 750ml of distilled water for 72 hr. Thereafter, the solution was filtered and the filtrate was evaporated to dryness in an oven at 40℃ to produce a dark brown residue. With respect to the powdered sample, the yield of the extract was 12.6%.

2.2 Experimental Animals

Healthy Wistar rats of both sexes weighing between 200 and 250 g were obtained from the Animal House of the College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were kept under normal laboratory conditions of temperature, humidity and light (12 hr day, 12 hr night). They were allowed free access to clean water and animal feed. The guideline and procedure for laboratory animal care was followed in handling the animals during the study (NIH, 1985).

2.2.1 Induction of experimental diabetes in rats

The rats were fasted overnight (12-14 hr) and their weight and fasting blood glucose levels were recorded. They were then made diabetic by a single intraperitoneal injection of alloxan monohydrate (120mg/kg body weight). Food and water were presented to the animals 30 min after the administration of alloxan (Nagappa et al., 2003). Five days after alloxan injection, blood samples were collected from the tail vein of the rats into heparinized tubes and plasma glucose levels of the animals were determined by glucose oxidative method (Manzella et al., 2001). Animals with fasting blood glucose ≥200 mg/dl were used for the study (Kumar et al., 2006).

2.3 Experimental Design

The rats were divided into two groups: non-diabetic rats and alloxan-diabetic rats. The hyperglycemic rats were divided into five groups. Each of the six groups consists of 5 animals. Group I = non-diabetic control; Group II = diabetic control; Group III = diabetic rats treated with KSE (50mg/kg); Group IV = diabetic rats treated with KSE (100mg/kg); Group V = diabetic rats treated with KSE (150mg/kg); Group VI = diabetic rats treated with glibenclamide (5mg/kg). All the drugs were administered orally once a day for 14 days. Groups I and II animals were fed orally with distilled water for the two weeks. Blood glucose levels and body weight were measured on day 0 (before treatment commenced), day 7 and day 14. Blood was drawn from the tail vein of the rats on these days and fasting blood glucose was measured by oxidative method as earlier described by Manzella et al. (2001). Oral glucose tolerance test and erythrocyte malondialdehyde (MDA) were estimated after the two week treatment.

2.4 Estimation of Erythrocyte MDA Concentration

The erythrocyte MDA concentration was determined by the double heating method (Draper and Hadley, 1990; Ambali et al., 2011). The color produced during the reaction of thiobarbituric acid (TBA) and MDA was measured spectrophotometrically. One milliliter of heparinized blood sample obtained from each rat was centrifuged at 600g. After discarding the plasma, erythrocyte packets were prepared by washing the erythrocytes three times in cold isotonic saline (0.9% w/v). The washed erythrocytes were then used for the estimation of MDA concentration. 2.5mL of 100g/L trichloroacetic acid was added to 0.5mL of erythrocytes in a centrifuge tube and placed in a boiling water bath for 15 min. The mixture was cooled in a tap water and centrifuged at 1000g for 10 min. Thereafter 2mL of the supernatant was added to 1ml of 6.7g/L TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water, and its absorbance was measured at 532nm. The concentration of MDA was calculated by the absorbance

coefficient of MDA-TBA complex, 1.56 × 105 cm-1 M-1, and expressed in nanomoles per gram of hemoglobin.

2.5 Oral Glucose Tolerance Test (OGTT)

After induction of experimental diabetes, the animals were made to fast for 12-14 hours. Their blood glucose level was measured by oxidative method and then the extract (150mg/kg) was given. Thirty minutes later, glucose solution (2g/kg body weight) was administered orally in a volume of 1ml. Blood samples were collected at 30, 60 and 120 min after the administration of glucose in order to evaluate blood glucose level.

2.6 Statistical Analysis

Values obtained as mean ± SEM were evaluated by one-way analysis of variance (ANOVA) followed by Scheffe's test using SPSS version 13. The values were considered to be significant when $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Body Weight

Alloxan-induced diabetic rats showed loss in body weight which was reversed by oral administration of aqueous extract of *Khaya senegalensis* (KSE). The body weight of non-diabetic control rats increased from 205.21 ± 6.93g on day 0 to 218.32 ± 10.46g on day 14 (6.38%). However the weight of diabetic control rats showed a significant decrease of 8.12% after two weeks. A dose-dependent body weight improvement was observed in diabetic rats treated with the extract. At a dose of 100mg/kg, there was an increase in body weight from 204.49 ± 12.02g on day 0 to 212.55 ± 13.45g (3.94% increase) on day 14, while 150mg/kg KSE increased the body weight from 220.86 ± 11.21g on day 0 to 231.64 ± 12.20g on day 14 (4.88% increase). The results are shown in table 1.

Table 1. Changes in body weight (g) of diabetic rats treated with aqueous extract of *Khaya senegalensis*

Group (n=5)	Day 0	Day 7	Day 14	% change
I	205.21± 6.93	207.31 ± 12.84	218.31 ± 10.46	+ 6.38
II	215.92 ± 9.36	204.34 ± 12.43	198.38 ± 8.34	- 8.12
III	223.86 ± 13.24	224.96 ± 9.26	226.22 ± 11.53	+1.05
IV	204.49 ± 12.02	210.92 ± 10.36	212.55 ± 13.45*	+3.94
V	220.86 ± 11.21	226.48 ± 11.24*	231.64 ± 12.20*	+4.88
VI	208.76 ± 14.22	214.41 ± 16.39*	220.94 ± 14.35*	+5.83

Each value represents mean ± SEM; % change indicates the change between day 0 and day 14.
**p<0.05 compared with diabetic control (group II)*

3.2 Fasting Blood Glucose Level

The values of fasting blood glucose level before (day 0) and after treatment for two weeks (day 14) in non-diabetic, untreated diabetic and treated diabetic rats are presented in table 2. The fasting blood glucose level did not show significant difference in the non-diabetic control.

In diabetic control, there was a significant difference ($p<0.05$) when compared with the non-diabetic control after two weeks of the experiment. With 50mg/kg of the extract, fasting blood glucose of diabetic rats was significantly decreased from 243.48 ± 12.44mg/dl to 185.60 ± 16.39mg/dl (23.77% reduction) between day 0 and day 14. 100mg/kg reduced fasting blood glucose from 235.30 ± 14.65mg/dl on day 0 to 168.42 ± 15.91mg/dl on day 14 (28.42% reduction), while 150mg/kg significantly reduced blood glucose from 228.83 ± 16.13mg/dl on day 0 to 146.45 ± 16.36mg/dl on day 14 (36.0% reduction). The standard drug, glibenclamide significantly lowered blood glucose of diabetic rats from 236.82 ± 16.33mg/dl on day 0 to152.80 ± 26.49mg/dl on day 14 (35.74% reduction).

Table 2. Effect of *K. senegalensis* extract on fasting blood glucose (mg/dl) in diabetic rats

Group (n=5)	Day 0	Day 7	Day 14	% change
I	112.06 ± 10.42	113.80 ± 10.44	111.64 ± 16.78	- 0.85
II	221.64 ± 9.35	268.86 ± 21.26	262.63 ± 28.50	+18.44
III	243.48 ± 12.44	238.50 ± 10.61	185.60 ± 16.39	-23.77
IV	235.30 ± 14.65	218.20 ± 18.30	168.42 ± 15.91	-28.42
V	228.83 ± 16.13	210.30 ± 16.40	146.45 ± 16.36	-36.00
VI	236.82 ± 16.33	221.56 ± 20.28	152.80 ± 26.49	-35.47

*Each value represents mean ± SEM; *p<0.05 compared with diabetic control. % change indicates the change between day 0 and day 14*

3.3 Oral Glucose Tolerance Test

The blood glucose level in the normal control rats rose to a peak value 60 min after the glucose load and decreased to near normal level at 120 min. In the diabetic control, the peak blood glucose level was also reached 60 min after glucose load but it remained high over the next 60 min. In the extract- treated diabetic rats, blood glucose level reached the peak at 60 min and then there was significant reduction ($p<0.05$) in blood glucose level as compared with diabetic control. This reduction in blood glucose level at 120 min was near the fasting blood glucose in rats treated with 150 mg/kg KSE. The results are presented in table 3.

Table 3. Effect of *K. senegalensis* aqueous extract on blood glucose levels (mg/dl) during OGTT in diabetic rats

Group (n=5)	0 min	30 min	60 min	120 min
I	108.30 ± 9.43	151.62 ± 13.51	139.82 ± 8.71	109.23 ± 8.50
II	218.42 ± 12.01	278.20 ± 18.23	290.60 ± 16.10	293.56 ± 21.06
III	229.83 ± 18.92	271.41 ± 12.33	264.25 ± 10.16	258.68 ± 10.18
IV	234.06 ± 10.66	265.54 ± 12.20	250.45 ± 13.38	236.37 ± 14.00*
V	226.29 ± 13.89	244.60 ± 12.84	236.36 ± 9.55	228.51 ± 11.35*
VI	238.12 ± 12.66	246.82 ± 13.44	243.48 ± 10.09	239.06 ± 9.68*

*Each value represents mean ± SEM; *p<0.05 compared with diabetic control*

3.4 Erythrocyte MDA Concentration

The erythrocyte MDA concentration in the diabetic control rats was significantly higher ($p<0.05$) compared to those obtained in the normal control and the treated groups. The MDA

concentration of diabetic animals was dose-dependently reduced by the extract. At 100 and 150 mg/kg, the reduction was significant ($p<0.05$) and was comparable to that observed with glibenclamide as shown in figure 1.

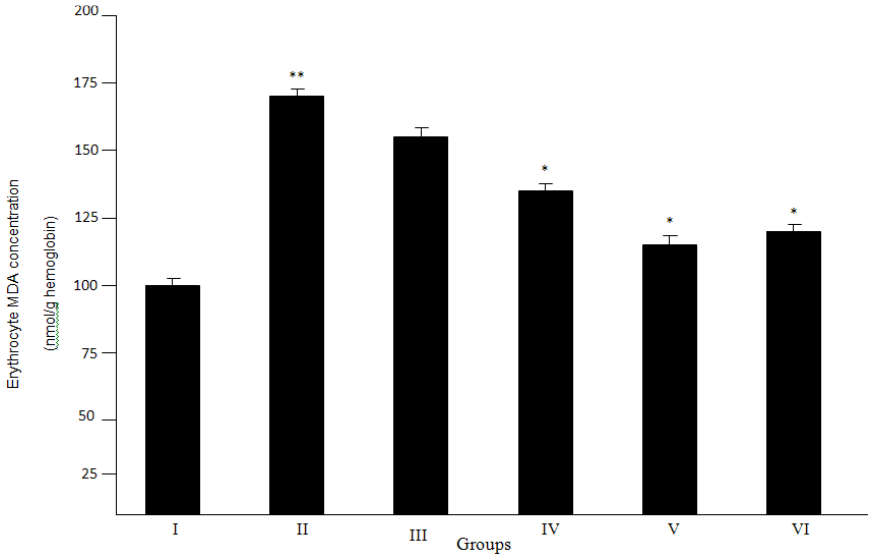

Fig.1. Effect of *Khaya senegalensis* (KSE) on erythrocyte MDA concentration in diabetic rats

*** p<0.05 compared with normal control; * p <0.05 compared with diabetic control*

The cytotoxic action of alloxan on pancreatic β-cells produces negative impact on insulin secretion and carbohydrate metabolism. This usually results in symptoms such as hyperglycemia, polyuria, polydypsia, ketonuria, ketoneamia, hepatic glucose overproduction and body weight loss (Milagro and Martinez, 2000). The decrease in body weight of diabetic rats observed in this study indicates excessive breakdown of tissue protein (Kamalakkannan and Prince, 2006). This is probably due to increased catabolic reactions leading to muscle wasting. Daily administration of *Khaya senegalensis* extract for 14 days to diabetic rats resulted in improved body weight. This suggests that the extract possesses antihyperglycemic effects which manifested as improved body weight in alloxan-induced diabetic rats (Pari and Saravanan, 2004). Treatment of diabetic rats with *K. senegalensis* extract brought down the elevated blood glucose level to nearly normal range in a dose-dependent manner. The extract also improved glucose tolerance in the rats. The blood glucose levels of the diabetic rats were in the range of 200-250 mg/dl which resembles type II diabetes that is often caused by lack of insulin sensitivity or resistance to insulin action at the receptor or post receptor level (Chaiken et al., 1993). Therefore *Khaya senegalensis* may have increased insulin sensitivity or decreased insulin resistance. In diabetes, reduced level of insulin increases the activity of fatty acyl coenzyme, resulting in lipid peroxidation. Increased peroxidation would lead to generation of harmful free radical, which impairs membrane function. This leads to cell injury and complications such as atherosclerosis, anemia, and kidney damage. Increased MDA concentration is an important indicator of lipid peroxidation (Ambali et al., 2011). The increased lipoperoxidation in the diabetic rats as reflected by the significant increase in the MDA concentration may promote the vulnerability of the red blood cells to destruction leading to anemia. Through the process of lipid peroxidation, the homeostasis and function of the erythrocyte membrane are impaired and

interaction and affinity of protein and lipids are altered (Dargel, 1992). This will eventually lead to cellular dysfunction and destruction. Aqueous extract of *Khaya senegalensis* dose-dependently caused significant reduction in lipoperoxidative damage to the erythrocyte membrane as demonstrated by the reduction in MDA concentration. Interestingly, aqueous extract of *Khaya senegalensis* has been demonstrated to possess anti-anemic effect on phenylhydrazine-induced anemia in rats at appropriate dosage (Sanni et al., 2005).

4. CONCLUSION

From the results of the study, hypoglycemic activities of the extract were comparable to those of the standard drug, glibenclamide. The present study has demonstrated that aqueous extract of *Khaya senegalensis* stem bark possesses hypoglycemic effect. Further investigations of the plant extract especially for its biologically active components and mechanism of action need to be carried out.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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