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Research paper

In vitro and in vivo studies of anti diabetic effect of khaya senegalensis leaves and bark extracts

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Abstract

Diabetes mellitus in Sudan is one of public health concern since it causes significant mortality and complications for long term. Though conventional drugs are used in the management of diabetes mellitus they are expensive, unavailable and also have numerous side effects. Khaya senegalensis has traditionally used in the management of diabetes. The present study was conducted to examine the In vitro and In vivo anti-diabetic activity of leaves and bark extracts of Khaya senegalensis. The leaves and bark of the plant were extracted with ethanol 96%, and then tested for anti-diabetic activity in a series of in vitro models and a type 2 diabetes model of rats. In vitro bark extract of k.senegalensis showed higher inhibitory activities against the enzyme with IC50 value 226.14 µg/ml. In vivo oral administration of the extracts of the k. senegalensis exhibited decrease in blood sugar level and was found to be time dependent. Bark extract showed strong in vitro and in vivo anti diabetic activity.

Keywords: *Khaya Senegalensis; Bark, Leaves; Anti Diabetic; A-Amylase; Rats.*

1. Introduction

Traditional medicine has been used for the treatment of human illnesses since long time and is mainly based on components derived from natural products obtained from herbs, plants, and animals. Due to their minor side effects, the medicinal plants are widely used to treat many human diseases. The increasing cost of health care and the failure of allopathic medicine to treat some diseases have also participated to the increasing consumption of traditional medicines to fight disease. Medicinal natural products are very frequently used in Sudan and also are widely consumed in Africa and all over the world. About 80% of the populations in African countries depend on traditional medicine for their primary health care (Maroyi, 2013). Khaya senegalensis A. Juss (Meliaceae) commonly called the dry zone mahogany or African mahogany is regarded as the most popular medicinal meliaceous plant in African traditional remedies (Danquah et al., 2012). The bitter stem bark aqueous extract have been used as a folk medicine for management of diabetes (Kolawole et al., 2012), hypertension (El Badwi et al., 2012), jaundice (Sule et al., 2008) and malaria (Bickii et al., 2010). Roots are applied topically against stomach ache, oedema and amenorrhoea (Ijeoma et al., 1997).

1.1. Diabetes mellitus

Diabetes mellitus could be defined as a heterogeneous group of a complex metabolic disorder associated with high blood glucose concentrations (hyperglycemia) and alterations in the metabolism of major macromolecules resulting from impairments in the secretion of insulin or its action. Diabetes is commonly accompanied with polydipsia, polyuria, microvascular problems involving eyes, kidney and peripheral nerves as well as cardiovascular problems such as hypertension. These complications affect about 50% of diabetic patients and can lead to their death thereby making diabetes a recognized fatal disease in different parts of the world for centuries. Insulin is a hormone synthesized by the β-cells at the pancreatic isles of Langerhans and its primary role is to tightly control blood glucose levels which usually rise after dietary intake. Thus, insulin is released from the pancreatic β-cells to normalize the glucose level. Therefore, in diabetic patients, abnormality in the production of insulin and/or its utilization causes hyperglycemia. Insulin is also vital to carbohydrates and lipids metabolism because it lowers blood glucose levels by enhancing the glucose uptake by cells and by stimulating glycogenesis as well as inhibiting glycogenolysis. It also retards the breakdown of fats to free fatty acids and ketone bodies. This hormone also encourages the storage of fat into the adipose tissues and reduces gluconeogenesis in liver and kidneys (Ibrahim et al., 2013).
Type II diabetes mellitus involve different pathways where the metabolic disorder was taken place as a result of either insulin resistance and/or insulin deficiency (Ibrahim et al.,2013). This medical condition consider as one of the most predominant type of diabetes since it represent 90% of diabetic cases. Moreover, this medical condition required a chronic monitoring and treatment throughout patient life; the treatment will involve several aspects like self-care measures, lifestyle changes (dietary modification) and in some cases medications

(Metformin and/or insulin). It has been observed during the past 50 years, the rate of incidence of this type of diabetes has markedly increased in parallel with obesity. This medical problem i.e., high blood sugar when remain for long time will mainly cause heart disease, strokes and diabetic retinopathy, all these will lead to renal failure (Hassan ,2013 and DeFronzo ,2004). The pathogenesis of T2D progress in two steps. In step one, normal individuals progress to impaired glucose tolerant (IGT) individuals with insulin resistance as the primary marker. Blood insulin levels are increased, and β-cell function is usually impaired. In the second step, IGT progresses to T2D due to a decline in β-cell function (DeFronzo, 2004).

2. Materials and methods

2.1. Preparation of plant materials

Dried leaves and bark of the plant materials of k.senegalensis were collected directly from the field at January 2018. The plant parts were thoroughly washed and air dried and ground to powder. One hundred gram of powdered material of leaves and bark were extracted with 96% ethanol for 72 hours at room temperature then filtered through Whatman number 4 filter paper. After filtration, the extracts were vacuum concentrated.

2.2. Methods

2.2.1. In-vitro antidiabetic activity of the ethanolic extracts of khaya senegalensis

Serial concentrations of test samples (leaves and bark extracts) and standard drug (Acarbose) (100-1000µg/ml) were prepared, then 500 µl of each prepared solutions were taken into another tests tube where 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α - amylase (0.5mg/ml) solution and incubated at room temperature for 10 min. After these, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at room temperature for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath at 100 ^{0}C for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm by Shimadzu Spectrophotometer UV double beam (Narkhede et al., 2011).
Calculation of 50% Inhibitory Concentration (IC_{50})
The concentration of the plant extracts required to inhibit 50% of the enzyme (α-amylase) (IC_{50}) were Calculated by using of Microsoft office Excel 2007 by the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition (%) was calculated by

% = (Ac-As)/Ac X 100

Where Ac is the absorbance of the control and as is the absorbance of the sample (Narkhede et al., 2011).

2.2.2. In-vivo antidiabetic of the ethanolic extracts of khaya senegalensis

2.2.2.1. Experimental animals and induction of diabetes

White albino rats (three to four weeks old) weighing 150-300 gm were considered in the study. Dry bread and water were used to feed the rats. Prior to the induction of diabetic state, the animals were fasted for about 18 hours. Intraperitoneal administration of 2.5g/mL of 50% glucose was used to induce diabetic state. Control cohort was administered with distilled water through oral rout. One hour post-induction of hyperglycemia, blood glucose was measured using a Glucometer (Kimani et al., 2017).

2.2.2.2. Experimental design

In the experiment, the rats were divided into four groups of five rats each. Group I rats (Negative control) were diabetic and were orally administered with 1 ml distilled water. Group II rats (Positive control) were diabetic and were orally administered with metformin (reference drug) at a dose of 500 mg/kg body weight. Group III and IV rats (Experimental groups) were diabetic and were orally given the plant extracts. Group III was given leaf extracts at doses 82.5, 82.5, 95, 110 and 75 mg/gm whereas group IV was given bark extracts at doses 130, 97, 102.5,100,150 mg/gm. The doses were given according to rats' body weight (Kimani et al., 2017).

2.2.3. Statistical analysis

Statistical Package of Social Sciences (SPSS) software was used to analyze the collected data. Results were expressed as the Mean ± standard deviation (SD) of the number of rats used per group.

3. Results

3.1. In-vitro antidiabetic activity of the ethanolic extracts of khaya senegalensis

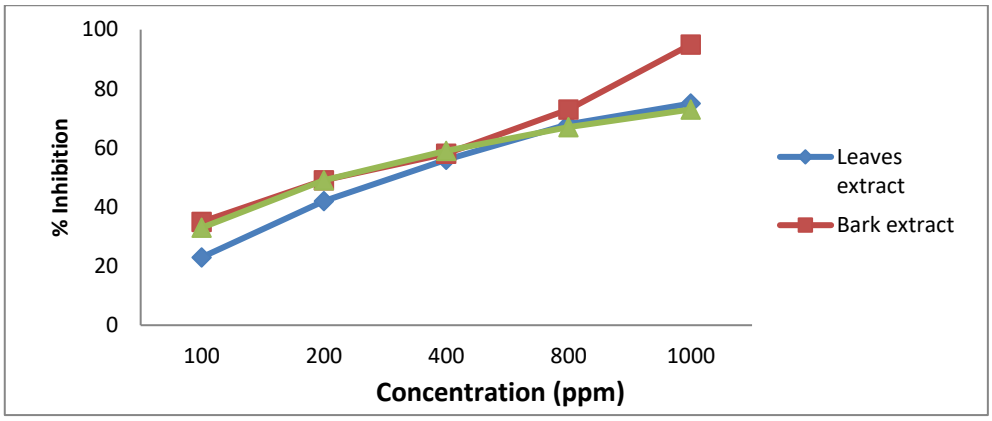

Fig. 1: A- Amylase Inhibition of Leaves and Bark Extracts of K.Senegalensis and Acarbose Standard Drug.

Table 1: IC_{50} of A- Amylase Inhibition by Leaves and Bark Ethanol Extracts of Khaya Senegalensis

Sample	IC_{50} µg/ml
Leaves extract	320.27
Bark extract	226.14
Acarbose (Control +ve)	250.65

3.2. In-vivo antidiabetic activity of leaves and bark ethanolic extracts of khaya senegalensis

Oral administration of the standard drug and ethanolic extracts of khaya senegalensis exhibited decrease in blood sugar level (Table 2&3, Figure 2) and results were found to be time dependent.

Table 2: Effect of Metformin Drug on Blood Glucose in Treated Diabetic Rat

	Descriptive Statistics				
	No. Rat	Minimum - Maximum		Mean ± SD	
		Control (-)	Metformin (+)	Control (-)	Metformin (+)
Rat body weight	5	192 - 248	170 - 260	216.60±25.589	208 ±37.848
blood glucose after fasting 18th hours	5	92 -122	69 - 135	105.20±12.911	107.40±28.077
Blood glucose after 1 houre taking glucose	5	96 - 252	84 - 353	152 ±58.975	152.40 ±113.487
Blood glucose after 2 hours	5	94 - 250	54 - 162	146.6 ± 59.902	91.40±44.134
Blood glucose after 4 hours	5	73 - 197	39 - 91	120.4±46.474	63.60±21.813
Blood glucose after 6 hours	5	60 - 168	23 - 80	99 ±41.467	47.80±23.026

Table 3: Effect of Leaves and Bark Extracts of Khaya Senegalensis on Blood Glucose in Treated Diabetic Rat

	Descriptive Statistics				
	No. Rat	Minimum - Maximum		Mean ± SD	
		Leaves	Bark	Leaves	Bark
Rat body weight	5	50 -220	195 - 300	158 ± 64.479	232 ± 46.179
blood glucose after fasting 18th hours	5	99 - 169	93 - 142	126.6 ± 27.69	115 ± 17.550
Blood glucose after 1 hour taking glucose	5	131 - 512	98 - 239	236.8 ±158.3	149.2 ± 55.061
Blood glucose after 2 hours	5	100 - 330	69 - 129	171.6 ± 96.111	99.6 ± 21.790
Blood glucose after 4 hours	5	71 - 201	61 - 107	112.6 ± 56.84	93.8 ± 18.847
Blood glucose after 6 hours	5	54 - 188	54 - 100	99 ± 55.929	88 ± 19.300

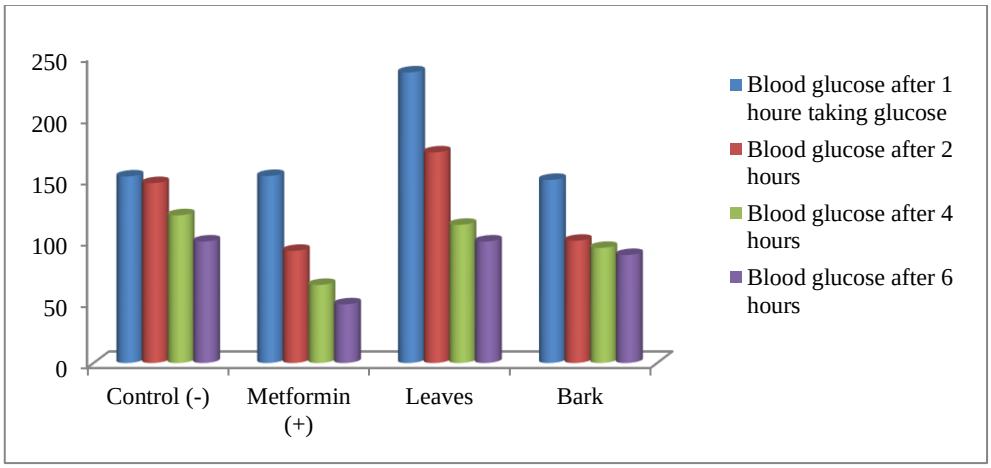

Fig. 2: Effect of Samples on Blood Glucose in Treated Diabetic Rats.

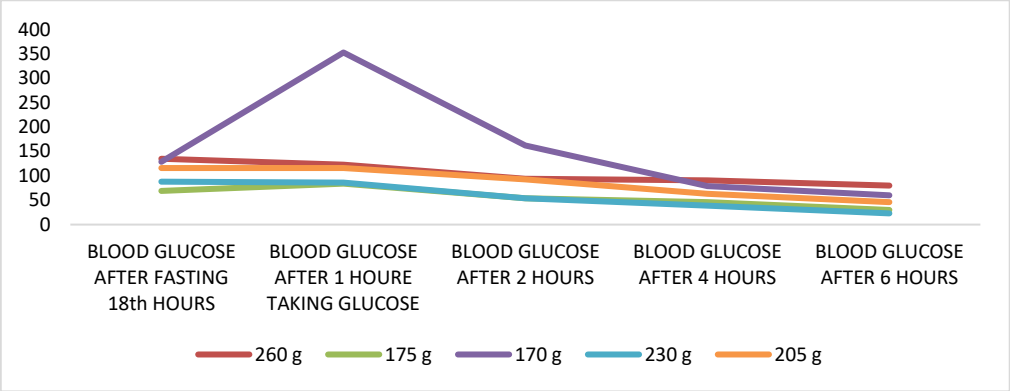

Fig. 3: The Relationship between Rat Weight and Blood Glucose Level for Control Positive Group.

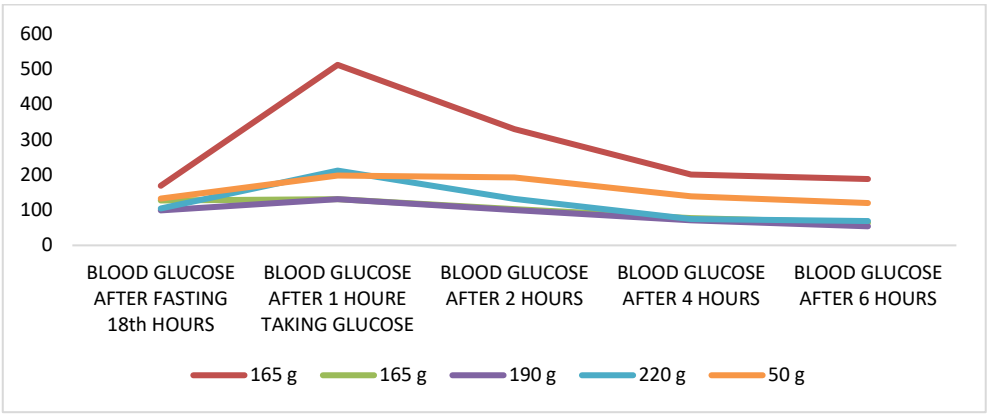

Fig. 4: The Relationship between Rat Weight and Blood Glucose Level for the Group Obtained Leaves Extract.

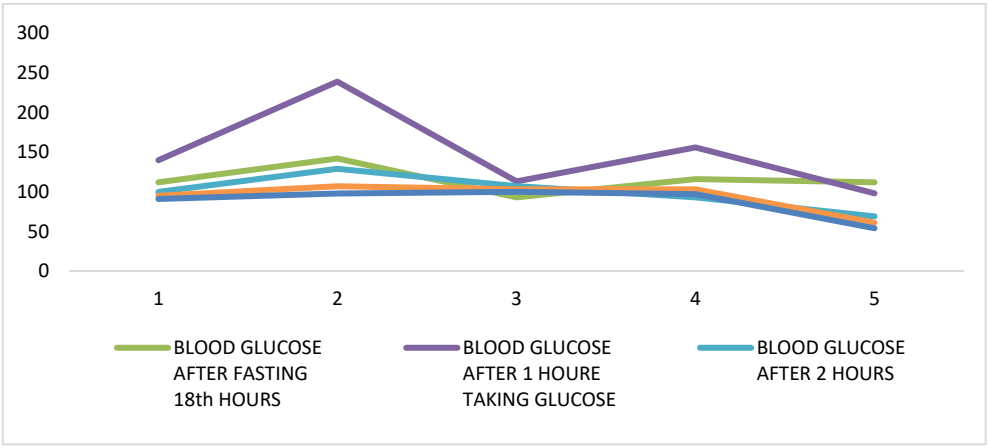

Fig. 5: The Relationship between Rat Weight and Blood Glucose Level for the Group Obtained Bark Extract.

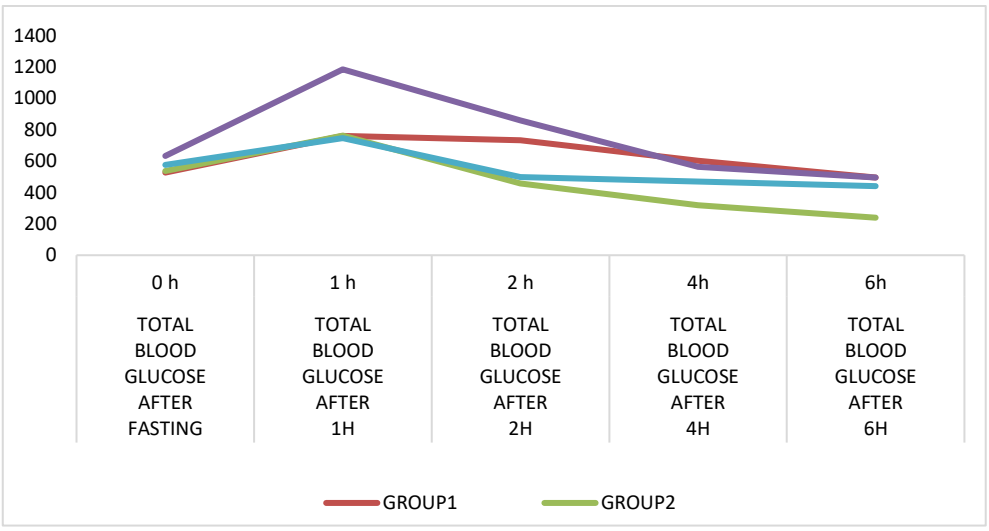

Fig. 6: Comparison between Rat Weight and Blood Glucose Level for All Groups in This Study.

(Group 1: control negative, Group 2: control positive, Group 3: leaves extract, Group 4: bark extract)

4. Discussion

Lack of insulin affects the metabolism of carbohydrates, proteins, fat and causes significant disturbance of water and electrolyte homeostasis. Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes and is more strongly associated with the risk for cardiovascular diseases than the fasting blood glucose. α-amylase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation (Davis and Granner, 2001). Alpha-amylase inhibitor retards the digestion of carbohydrates and slows down the absorption. Acarbose and miglitol are competitive inhibitor of α-glucosidases and reduces absorption of starch and disaccharides. Hence one of the therapeutic approaches for reducing postprandial (PP) blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of these enzymes (α-amylase and α-glucosidases) reduced the high postprandial (PP) blood glucose peaks in diabetes the α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Bark extract of khaya senegalensis maintained higher inhibitory activities against the enzyme as shown by the IC_{50} value 226.14 µg/ml as shown in Table 1. This result may be due to the presence of higher phenolic compounds (Widdat Allah et al., 2018). Where they reported a strong positive correlation between polyphenolic content and α-amylase inhibitory effects of 28 extracts from Vietnamese edible plants but also suggested that the enzymatic inhibition might depend on the type of the polyphenolics (Ibrahim et al., 2013).

Khaya senegalensis is traditionally used in the management of diabetes in some West African countries. In this study the rats induced diabetes by administration of glucose 50% to have much higher blood sugar level compared to the glucose level before administration (Figure 3, 4, 5, 6). Significant difference in the group given leaves extract was observed while no difference in the group given bark extract which can be due to high rat's weight for this group which can produce resistance in decreasing blood sugar level (Ibrahim et al., 2013).

The blood sugar lowering effect could be associated to Saponins which were found to be present in ethanolic extracts of khaya senegalensis. Saponins have been demonstrated to have antidiabetic effect. Khaya senegalensis ethanolic extracts contain tannins which are known to have the antidiabetic activity (Kimani et al., 2017).

5. Conclusion

The present study confirm the traditional uses of Khaya senegalensis as antidiabetic medicinal plant by some Sudanese people in Sudan. The results indicated that bark and leaves extracts of K.senegalensis reduce the glucose level in diabetic rats. The result showed high antidiabetic activity in bark extract and it was very closed with that obtained by metformin standard drug, while leaves extract has weak antidiabetic activity. Further investigations are warranted to identify the active principles and elucidate other possible mechanism of action.

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