Antidiabetic activity of the methanol leaf extract of *Commiphora Kerstingii* Engl. on experimental hyperglycaemia in Wistar Rats

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ABSTRACT

Diabetes mellitus, simply referred to as diabetes, is a syndrome characterized by disordered metabolism and inappropriately high blood sugar (hyperglycaemia) resulting from either low levels of the hormone insulin or from abnormal resistance to insulin's effects coupled with inadequate levels of insulin secretion to compensate. Commiphora kerstingii is a tree of 10m high that grows in savanna from Togo to Nigeria, and on to Central African Republic, and it belongs to the family Burseraceae. In Zaria Nigeria, the leaf is used traditionally for managing diabetes mellitus. Phytochemical screening was carried out in accordance with the standard protocol as described by Trease and Evans and the oral LD_{50} of the extract in rats was conducted according to the method described by Lorke. Glucose, nicotine and dexamethasone were used to in induce hyperglycaemia. The dose of 400 mg/kg of the extract significantly ($p < 0.02$) prevented an elevation in blood glucose at the 2nd and 3rd hours in the oral glucose load model. In the nicotine-induced hyperglycaemic model 100 mg dose of the extract significantly ($p < 0.05$) lowered the BGL only at the 5th hour, while 200 mg/kg and 400mg/kg doses lowered the BGL at the 4th and the 5th hours. Dexamethasone was administered subcutaneously at a dose of 10 mg/kg/day for ten days consecutively. All doses of the extract used significantly ($p < 0.05$) lowered the Blood Glucose Level when compared with the group that was administered dexamethasone alone on all the days monitored.

Keywords: *Commiphora kerstingii,* glucose, nicotine, dexamethasone, hyperglycaemia
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INTRODUCTION

Medicinal plants have been used for treatment of various conditions since time immemorial; they are believed to be important sources of new chemical substances with potential therapeutic effects [1; 2]. There is presently an increasing interest in herbal medicine. The entire plant, roots, stem bark, leaves, fruit, seed or juice are employed for various traditional practitioners; sometimes inappropriately. There has been the need for several years now for safer, more effective and cheaper antidiabetic drugs. Diabetes is the most common endocrine disorder. More than 150 million are suffering from it worldwide [3], and its likely to increase to 300 million by the year 2025.

It is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin [4]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations.

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The increasing prevalence of diabetes has reached epidemic proportion worldwide, and it is a major threat to global health that is rapidly increasing [5]. The greatest increase in prevalence is however expected to occur in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence in diabetes in developing countries follows the trend of urbanization and lifestyle changes.

Dexamethasone at a dose of 2mg/kg intraperitoneally was used to induce hyperglycaemia in Sprague Dawley halothane anaesthetized rats [6]. Four days after administration, the plasma glucose level was observed to be 159% greater than in normal rats. Nicotine has been shown to stimulate the adrenal gland resulting in the discharge of adrenaline. The rush of adrenaline stimulates the body and causes a sudden release of glucose.

Commiphora kerstingii is a tree of 10m high that grows in savanna from Togo to Nigeria, and on to Central African Republic, and it belongs to the family *Burseraceae*. The wood is soft it is used to make saddle and is sometimes hollowed out to make quivers in Yola area of Northern Nigeria. The evergreen bark seems to have engendered an idea that the tree is little likely to burn, so that it has acquired vernacular names suggestive of protection against fire, and survival of property and therefore of inheritance. Traditionally the leaf is used by people of North Western Nigeria for managing diabetes mellitus. The aim of the study is to scientifically evaluate the antidiabetic effect of the leaf extract of *C. kerstingii* in Wistar rats.

MATERIALS AND METHODS

Plant preparation and experimental animals
The fresh leaves of *Commiphora kerstingii* was collected in Samaru, Zaria, Kaduna State of Nigeria in the month of November. The plant was authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Kaduna state, Nigeria. A voucher specimen number of 006 were deposited at the herbarium for future reference.

Male and female Wistar rats weighing 150-200g were used in the study; they were obtained from the Department of Pharmacology and Toxicology Usmanu Danfodiyo University Sokoto. The animals were maintained on standard laboratory animal feed and water *ad libitum,* and housed in polypropylene cages at room temperature and a 12 h light-dark cycle throughout the study. These studies were carried in accordance with the rules governing the use of laboratory animals as accepted internationally [7].

Preparation of the plant extract
The leaves were washed and air-dried under shade until a constant weight was obtained on three separate weighing and then size-reduced into powder with a pestle and mortar. About 100g of the powdered leaf was macerated with 500ml methanol for 72hour with occasional shaking. The extract was concentrated *in vacuo* and subsequently referred to as methanolic leaf extract of *Commiphora kerstingii*. Solutions of the extract were prepared freshly for each study.

Phytochemical screening
The screening was carried out in accordance with the standard protocol as described by [8].

Acute toxicity study
The oral LD_{50} of the extract in rats was conducted according to the method described by [9]. Briefly, the method was divided into two phases. In the initial phase, animals were randomly divided into 3 groups of three rats each. Group I, II and III were treated with 10, 100 and 1000 mg/kg body weight orally of the extract and observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one mouse was administered with four more specific doses of the extract based on the results obtained during the first phase. The LD_{50} value was calculated by taking geometric mean of the lowest dose that caused death and the highest dose that did not produce death.

Oral Glucose-induced Hyperglycaemia Model
In this model described by [10], 12- 14 h fasted rats were randomly divided into 5 groups of 5 rats each. Group V served as the model control and were pretreated with 1mg/kg glibenclamide 1 hour before the oral administration of 3 g/kg of D-glucose. Groups I – IV were administered 10ml/kg/oral distilled water, 100 mg/kg, 200 mg/kg and 400 mg/kg of methanol leaf extract of *C. kerstingii* respectively, 1 hour before treatment with 3 g/kg/oral D-glucose.

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The blood glucose level of the animals was recorded at 0 hour and then after every one hour for the following six hours using an acucheck glucometer with compatible strips. A drop of blood was collected from the tail tip of the animals.

Nicotine-induced Hyperglycaemia Model
The same experimental procedure as described above was used for this model, but nicotine 50ug/kg was administered intraperitoneally instead of glucose as described by [11].

Dexamethasone Induced Insulin Resistance Model
For this model described by [12, 13, and 14], thirty-six male rats were divided into 5 groups of 5 animals. The grouping is as follows:

Group I: Dexamethasone sodium phosphate 10 mg/kg, once daily/SC + Normal saline
Group II: Dexamethasone 10 mg/kg/SC + 100 mg/kg/oral of methanol leaf extract of *T. indica*
Group III: Dexamethasone 10 mg/kg/SC + 200 mg/kg/oral of methanol leaf extract of *T. indica*
Group IV: Dexamethasone 10 mg/kg/SC + 400 mg/kg/oral of methanol leaf extract of *T. indica*
Group V: Dexamethasone 10 mg/kg/SC + 1mg/kg glibenclamide

Animals in all the groups were treated daily for ten consecutive days. The blood glucose levels, was recorded on the 1st, 3rd, 6th, 8th, and 10th days.

RESULTS

Table 1. Mortality and percentage mortality of the doses of *C. kerstingii* extract administered orally in Wistar rats during the first phase of the acute toxicity study.

Group (n = 3)	Treatment	Mortality	% Mortality
1	Control normal saline	0/3	0.00
2	10 mg/kg extract	0/3	0.00
3	100 mg/kg extract	0/3	0.00
4	1000 mg/kg extract	0/3	0.00

Table 2. Mortality and percentage mortality of the doses of *C. kerstingii* extract administered orally in Wistar rats during the second phase of the acute toxicity study.

Group	Treatment (mg extract / kg)	Mortality	% Mortality
1	1,600	0	0.00
2	2,900	0	0.00
3	5,000	0	0.00

The oral acute toxicity test (LD_{50}) of the extract was found to be greater than 5,000 mg/kg body weight.

Phytochemical test of the methanol leaf extract of *Commiphora kerstingii* Engl. showed the extract to contain carbohydrate, saponins, tannins, flavonoids, alkaloids, and steroids.

Table 3: Effect of methanolic leaf extract of *C. kerstingii* on Oral Glucose-induced hyperglycaemic Wistar rats

Treatment	Time (hr)					
	0	1	2	3	4	5
Normal saline	73.4±4	77.0±4	130.3±13	160.3±13	108.5±1	105.0±2
CK 100mg/kg	79.0±2	82.0±2	93.0±5	79.8±3*	97.3±6	76.3±4*
CK 200mg/kg	74.5±4	77.5±4	76.5±4*	82.8±3*	84.5±2*	85.8±3*
CK 400mg/kg	78.5±2	83.8±2	82.8±2**	84.8±3**	83.0±4*	81.0±4**
Glibenclamide 1mg/kg	78.3±6	80.3±6	79.3±6*	77.5±5**	76.5±5**	74.5±5**

*n = 5 * = significant at p < 0.05 Vs Normal saline (group I); Student's T-test ** = significant at p < 0.02 Vs Normal saline (group I); CK = Commiphora kerstingii*

The dose of 400 mg/kg of the extract significantly ($p < 0.02$) prevented an elevation in blood glucose at the 2nd and 3rd hours. The 200 mg/kg and 100mg/kg doses of the extract significantly ($p < 0.05$) prevented a rise in the blood

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glucose from the 2nd to the 5th hour. The standard drug (glibenclamide) significantly ($p < 0.02$) lowered the blood glucose level at the 3rd, 4th and 5th hours (table 3).

The 200 mg/kg and 400mg/kg doses lowered the BGL at the 2nd, 4th and the 5th hours. The standard agent used (glibenclamide) used significantly ($p < 0.02$) lowered the BGL at the 4th and 5th hours (table 4).

Table 4:   Effect of the methanol leaf extract of *C. kerstingii* on nicotine-induced hyperglycaemic Wistar rats

Treatment	Time (hr)					
	0	1	2	3	4	5
Normal saline	118.5±2	108.5±2	127.5±2	116.5±2	105.5±2	110.5±2
CK 100 mg/kg	98.3±6	99.8±6	98.8±6	93.3±6	90.3±6	98.3±6
CK 200 mg/kg	74.3±4	75.5±4	86.3±4	91.3±4	88.3±4*	86.3±4*
CK 400 mg/kg	74.5±4	76.0±4	85.0±4**	90.0±4	87.0±4*	85.0±4*
Glibenclamide 1mg/kg	79.0±2	78.5±2	83.6±2**	87.3±2**	76.3±2**	73.3±2**

n = 5; * = significant at p < 0.05 Vs Normal saline (group I); Student's T-test ** = significant at p < 0.02 Vs Normal saline (group I)
CK = Commiphora kerstingii

Dexamethasone was administered subcutaneously at a dose of 10 mg/kg/day for ten days consecutively. In table 5 all doses of the extract used significantly ($p < 0.05$) lowered the Blood Glucose Level (BGL) when compared with the group that was administered dexamethasone alone on all the days monitored. Glibenclamide the standard agent used significantly ($P < 0.02$) lowered the BGL.

Table 5: Effect of methanol leaf extract of *T. indica* on blood glucose on dexamethasone- induced hyperglycaemia

Treatment	Days				
	1	3	6	8	10
Normal saline	88.0±0	107.3±1	114.3±1	130.8±3	112.0±1
CK 100 mg/kg	88.0±1	90.0±1*	95.0±1*	95.0±1*	98.0±1
CK 200 mg/kg	82.0±0	84.8±3*	89.8±3*	89.8±3*	92.8±3*
CK 400 mg/kg	88.0±2	90.0±2*	96.0±2*	96.0±2*	99.0±2
Glibenclamide 1mg/kg	83.0±6	90.0±5*	89.0±6**	89.0±6**	91.0±3*

n = 5; * = significant at p < 0.05 Vs Normal saline (group I); Student's T-test ** = significant at p < 0.02 Vs Normal saline (group I)
CK = Commiphora kerstingii

DISCUSSION

In this study, the investigators showed the ability of the leaf extract of *Commiphora kerstingii* Engl. in lowering an elevated blood glucose concentration as well as investigated the efficacy of the extract at preventing a rise in blood glucose level.

There are several reports shownning that phenolic compounds (e.g. tannins, coumarins, and flavonoids), triterpenoids and a host of other secondary metabolites posses' hypoglycaemic effects in various experimental animals [15, 16, and 17]. Phytochemical screening of the methanolic leaf extract of *Commiphora kerstingii* showed the presence of carbohydrate, glycosides, cardiac glycosides, steroids and terpenoid, flavonoids, tannins and alkaloids. The antihyerglycaemic effect seen with this extraxt could be attributed to the presence of one or combination of the active chemical constituents in the extract.

An acute toxicity study in animals is important to drug development. In most cases the study tries to establish a precise median lethal dose (LD_{50}) in laboratory animals. The oral median lethal dose of methanol leaf extract of *Commiphora kerstingii* in rat was found to be greater than 5,000 mg/kg. This suggests that the leaf extract is non-toxic when administered orally in Wistar rats.

The post absorptive state of glucose is marked by postprandial hyperglycaemic state which is often accompanied by increased pancreatic insulin secretion, particularly in the first few hours postprandial [18]. In this study, graded doses of the extract prevented this increase and at the 5th hour lowered the glucose concentration close to the level at zero hour. The 100 mg/kg and 200 mg/kg doses significantly ($p < 0.05$) prevented the elevation from the 3rd to the 5th hours. Glibenclamide gave significant ($p < 0.02$) reduction from the 3rd to 5th hours. It is hereby suggested that the extract produced its hypoglycaemic effect by inhibiting intestinal glucose uptake like the α-glucosidase inhibitors.

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Dexamethasone stimulates lipolysis and free fatty acids synthesis which may compete with glucose for intracellular glucose oxidation, leading to insulin resistance through the glucose–fatty acid cycle, [19, 20, 21]. In the present study, dexamethasone administration for 10 days resulted in increased BGL when compared to group I animals (untreated control) similar to a previous study by [22, 3]. The BGL was significantly ($p < 0.05$) reduced in all the doses and with glibenclamide.

Nicotine has been shown to cause an increase in BGL in rats [23, 10] and canines [24, 25]. Nicotine is known to stimulate the nicotinic acetylcholine receptors [26]. In this study hyperglycaemia was induced by administration of 50ug/kg of nicotine to the animals. However, the hyperglycaemic state was attenuated by all the doses of the extract used and the standard agent by the 5th hour. *C. kerstingii* could have mediated this effect by the inhibition of adrenergic homeostatic mechanism

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