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Research Article

Evaluation of antidiabetic activity of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) leaf and bark in alloxan induced diabetic rats

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ABSTRACT

The study aimed to evaluate the antidiabetic effect of *Irvingia gabonensis* leaf and bark extracts on alloxan induced diabetic rats. Forty-four healthy rats were used in the experiment; they were grouped into eight groups. Alloxan was injected intraperitoneally and increase in the blood sugar level was confirmed after three days with glucometer (AccuCheck). The aqueous and ethanolic extracts of both leaf and bark at 150 and 250 mg/kg were orally administered daily for 16 days. Blood sugar level and body weight of each group were monitored and haematological parameters determined at the end of the experiment. Diabetic groups to which aqueous leaf extract at doses of 150 mg/l and 250 mg/l were administered showed significant decrease in blood sugar level from 463.50±29.32 and 381.33±39.77 to 103.50±4.50 and 97.00±2.00 respectively, compared to the positive group treated with glibenclamide, 468.67±30.82 to 86.67±2.73 ($P<0.05$). The body weight showed significant increased after the 8th day in group treated with aqueous leaf extract at dose of 150 mg/l and 250mg/l (191.25±16.84 to 207.7±37.90 and 147.35±0.85 to 150.60±1.20) when compared to the positive control group (164.07±5.76 to 171.57±4.81) at $P<0.05$. Aqueous extracts of leaf and bark of *Irvingia gabonensis* had more anti-diabetic activity than the ethanolic extracts. They also improved the haematological indices of the treated animals.

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1. INTRODUCTION

Plants have, over several years been good sources of drugs and there are ethno-botanical reports on more than 800 plants [1]. The beneficial uses of medicinal plants in traditional medicine of many cultures are extensively documented [2]. Although, there are many orthodox medicines produced for the cure of several diseases such as diabetes, malaria, jaundice, which have been proved effective, but complications or side effects of these drugs have been reported [3-6].

One of many diseases afflicting human generation over the years is diabetes, which has caused loss of life in both young and old [7-9]. As reported, diabetes is a common and globally among top three diseases that kill people throughout the world and has gained popular attention [10]. Diabetes is a group of metabolic disorders with a common manifestation hyperglycaemia i.e. increase in blood sugar level [5,7,10-12].

The first line treatment for diabetes is usually diet and exercise and sometimes these measures alone are sufficient to bring blood glucose levels back to the normal range. If these measures do not effectively control blood glucose levels, one or a combination of medications may be necessary to control hyperglycaemia [13]. The medications for diabetes come in various classes; each class contains one or more specific drugs. Some of these drugs are taken orally. Others must be injected. Various diabetes drugs work in different ways to lower blood sugar. A drug may work as stated by [13] by stimulating the pancreas to produce and release more insulin;

by inhibiting the production and release of glucose from the liver; or by blocking the action of gastric enzymes for carbohydrate catabolism or make tissues more sensitive to insulin.

In view of undesirable side-effects of orthodox therapies, the search for alternative medicines has been on to find out ways to avoid some of these by using drugs that are equally effective but with little or no side-effects [13]. Hence, the use of botanical medicines became the major concern. Although, treatments of diabetes mellitus with the use of plants have been in existence for many years [14], traditional anti-diabetic plants might provide new oral anti-diabetic compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing countries [10]. Interestingly, the parent compound of biguanides used in the treatment of insulin resistance was originally isolated from a plant source (French lilac) [15]. Consequently, this research showed antidiabetic activities of *Irvingia gabonensis* leaf and bark in Alloxan induced diabetic *r*ats

2. MATERIALS AND METHODS

2.1 Plant collection and processing

Fresh leaves and stem bark of *I. gabonensis* were collected from a farm land in Ado Ekiti due to their large cultivation and familiarity of the area. The collections were authenticated at the Department of Botany, University of Ibadan, Nigeria. The leaves and stem bark of *I. gabonensis* was air-dried and pulverized into fine powder with a domestic electric grinder, sieved and packaged into polythene bag and stored in dry conditions until when required for use.

Extraction was carried out by weighing fifty grams (50 g) of leaf and stems bark powder separately and each was soaked in 250 ml of distilled water and ethanol. It was stirred at every one hour (1hr) for 3hours. This was allowed to stand for 48 hours at room temperature (28±2 ^{o}C). At the end of the extraction, the extract was filtered using Whatman No 1 filter paper. The filtrate was collected in a beaker and concentrated using a Hot plate oven set at 35 ^{o}C until it gave a gelatinous residue. Plant extracts of both aqueous and ethanolic leaf and bark were collected and stored in clean sterile containers for further use.

2.2 Experimental animal and experimental set up

Sixty-five healthy Wistar adult albino rats of 12 males and 53 females were obtained from the Department of Biochemistry, Federal Polytechnic, Ado - Ekiti. The animals were transported to University of Ibadan and kept in clean cages in the animal house of Department of Zoology under standard conditions and were fed with standard pellets and water *ad libitum* for 14 days to acclimatize. After the 14 days acclimatization, the animals were weighed; body weight of 120 g and above were considered to be used in the experiment. Out of 65 animals, forty-four (44) weighed 120g and above, in which 8 were males and 36 females, the rats were grouped into eleven (11) groups of four (4) rats placed in each group: groups 1 (non diabetic normal control), 2 (negative control; no treatment), 3 (positive control; glibenclamide treatment), 4 (diabetic rats administered orally with 150 mg/kg of aqueous leaf extract), 5 (diabetic rats administered orally with 250 mg/kg of aqueous leaf extract), 6 (diabetic rats administered orally with 150 mg/kg of aqueous bark extract), 7 (diabetic rats administered orally with 250 mg/kg of aqueous bark extract), 8 (diabetic rats administered orally with 150 mg/kg of ethanol leaf extract), 9 (diabetic rats administered orally with 250 mg/kg of ethanol leaf extract), 10 (diabetic rats administered orally with 150 mg/kg of ethanol bark extract) and 11 (diabetic rats administered orally with 250 mg/kg of ethanol bark extract).

2.3 Inducing of diabetes and analysis of blood samples

The animals were starved overnight and administered a single dose of alloxan monohydrate, intra-peritoneally (150 mg/kg body weight) in saline at 0.9% obtained commercially (alloxan monohydrate product of Sigma-Aldrich Co. USA) [16]. To determine the diabetic level of each induced rat, after 4 days blood samples were collected at the tail tip with the aid of dissecting kit. The strip of the Glucometer was inserted into the instrument (AccuCheck glucometer) and a drop of the blood sample collected was placed on it for the reading. Animals with blood sugar level of 200mg/dl and above were exposed to the treatment. The animals in group 4 to 11 were orally administered aqueous and ethanolic extracts of the leaf and bark of *I. gabonensis* at 2 ml of 150 and 250 ml/kg/body weight/ rat/day while group 3 were also administered orally glibenclamide at 2 ml of 5 mg/kg/ body weight/ rat/day for 16 days. The collection of blood samples were done at 1st, 4th, 8th, 12th and 16th days post treatment to determine the blood sugar level and values recorded. After 16 days, the animals were sacrificed under anaesthesia and blood was collected through cardiac puncture for haematological analysis [17].

2.5 Statistical analysis

Analysis of Variance (ANOVA at $P < 0.05$) was used to determine the variations in the mean values of fasting blood level before and after treatment with extracts in the diabetic groups as well as between the various dose levels and control for each sampling period.

3. RESULTS AND DISCUSSION

The body weight of the animals at 0 day (pre-induction) showed that there was no significant

difference ($P < 0.05$) in the experimental groups compared to positive control group but there was significant difference within the treated groups. Group 4 (aqueous leaf extract at 150 mg/l) was significantly higher, as presented in Table 1.

There was significant weight loss observed in all the diabetic groups on day 4, including the positive control group. On day 8, significant weight gain was observed in diabetic groups treated with aqueous bark extract at 250 mg/kg or glibenclamide. There was no survival in diabetic group to which ethanolic leaf at 150 mg/l dose was administered and the negative control while only one animal survived in diabetic group to which ethanolic bark extract at 150 mg/l was given.

However, on day 12 to 16, significant weight gain was observed in the diabetic groups treated with aqueous leaf (150 and 250 mg/kg), aqueous bark (250 mg/kg) and glibenclamide. There was no surviving animal in diabetic group administered with ethanolic bark extract at 250 mg/kg while in diabetic groups treated with aqueous bark at 150mg/kg and ethanolic leaf at 250 mg/kg extracts respectively had one surviving animal each.

Weight loss was observed on day 4 in the experimental groups may be attributed to loss of appetite due to the effect of alloxan. On day 8 till day 16, however, the effect of the aqueous leaf extracts (150 and 250 mg/kg) observed on the body weight revealed significant weight gain. This observation indicated that the leaf and bark aqueous extracts of *Irvingia gabonensis* possessed the ability of managing glucose level as well as controlling muscle wasting and induce adipogenesis, thereby increasing the body weight. This is in agreement with (18) that reported the long-term antidiabetic and anti-hyperlipidaemic effects of aqueous stem bark extract of *Irvinga gabonensis* in streptozotocin-induced diabetic rats in which there was increase in body weight of induced animals after two weeks of treatments with extract at 200mg/kg, it also agrees with [19] who reported the aqueous extract of stem bark of *Afzelia africana* (Smith) resulted in increased body weight of diabetic rats and [9] who reported that soybean seeds to manage the body weight of the alloxan-induced animals [20] also reported that leaf extract of *Phyllanthus amarus* improved body weight of alloxan-induced diabetic mice. The significant decreased observed in body weight of surviving animal in ethanolic leaf and bark group could be attributed to the toxicity effect of the extract on the metabolic activities of the animals' body system and inability to control muscle wasting. This was in agreement with [21] who reported that plant extract have reduction effect on cholesterol in the body. The mortality observed in ethanolic extract of both leaf and bark groups at 150 and 250 mg/kg respectively could be as a result of the animals inability to recover from the effect of alloxan on their pancreas with given doses and also, this may be suspected to be as a result of toxicity of the given ethanolic extracts which prevented the production of insulin from the existing β cells, this was in agreement with work done by [22] who investigated the effect of Leaf extract of *Irvingia gabonensis* on Urine output and Electrolytes in Rats reported that the urine output of the animals were increased with amount of dose given. It has also been established that polyuria is one of the symptoms of diabetes which can as well lead to body weight reduction. Although, this does not agree to some extent with [6] who reported the prophylactic effect of ethanolic extract of *Irvingia gabonensis* stem bark against cadmium-induced toxicity in albino rats which led to weight gain of the body.

At pre-induction Day 0, there were no significant differences observed in blood sugar level of the experimental groups compared to normal control group ($P < 0.05$), which significantly increased on day 4 post-induction among the diabetic groups, in comparison with normal control group.
On day 8, significant decrease in blood sugar level was recorded in groups administered aqueous leaf and bark extracts at 150 and 250 mg/kg or glibenclamide and positive control. However, groups administered ethanolic leaf extract at 250 mg/kg and the negative control could not recovered from increased blood sugar level, which resulted to mortality. Increased blood sugar level was observed in groups treated with ethanolic bark extract at 150 and 250mg/kg, with values too high for the glucometer sensitivity, though they were alive.

On days 12 and 16, there were further significant decreases in blood sugar in groups treated with 150 and 250 mg/kg doses of aqueous leaf extracts. The reduction in blood sugar level was more pronounced in diabetic group administered with aqueous leaf extract at 250 mg/kg in which the value was reduced to normal blood sugar level range while that of aqueous bark extract (250 mg/kg) was near normal. However, diabetic groups to which aqueous bark and ethanolic leaf extracts at 150mg/kg were administered had only one survival each at this point. On day 12, the performance of ethanolic bark extract at 250mg/kg was observed to reduce blood sugar level of the surviving animal in the group to a detectable value and there was further reduction observed on the 16th day.

On day 16, the performance of aqueous leaf (150 and 250 mgkgl) and glibenclamide administered, consequently, reduced the blood sugar level of the diabetic animals to normal when compared to the normal control group, while diabetic group to which aqueous bark extract (250 mg/kg) was administered was close to normal. However, the diabetic groups to which ethanolic extracts were administered, had one survival each in the groups given 250 mg/kg of leaf extract and 150mg/kg of bark extract while there was no survival in the diabetic

groups given 150 mg/ kg of leaf and 250mg/ kg of bark. This observation suggested that the ethanolic extracts could have toxic effect on the animals' body system. This could be an indication that most of the aqueous extracts are more active than the ethanolic extracts.

Table 1. Effect of aqueous and ethanolic leaf and bark extract of *I. gabonensis* on body weight of Alloxan-induced diabetic Wistar rats.

Treatment	Solvent	Dose (mg/kg)	Mean Body Weight (g)				
			0 day (Pre-Induction)	4th day	8th day	12th day	16th day
Leaf	Aqueous	150	197.33±16.66^{b}	192.78±15.26^{c}	191.25±16.84^{a}	204.80±36.30^{a}	207.7±37.90^{a}
		250	149.60±6.81^{a}	148.30±6.44^{abc}	147.35±0.85^{a}	153.70±5.62^{a}	150.60±1.20^{a}
	Ethanol	150	149.68±10.34^{a}	145.95±10.04^{abc}	NS	NS	NS
		250	181.13±4.57^{ab}	180.53±4.46^{abc}	177.95±9.55^{a}	183.90±0*	178.10±0*
Bark	Aqueous	150	180.58±4.09^{ab}	179.25±3.60^{abc}	177.90±3.41^{a}	182.90±0*	183.20±0*
		250	146.85±17.30^{a}	142.18±18.71^{ab}	162.65±27.85^{a}	163.40±27.80^{a}	164.30±27.60^{a}
	Ethanol	150	137.40±4.96^{a}	133.33±3.94^{a}	130.60±0*	128.20±0*	123.90±0*
		250	181.13±5.26^{ab}	170.85±0.15^{abc}	164.15±1.15^{a}	NS	NS
- control (NT)	-	-	155.15±9.61^{ab}	151.60±9.04^{abc}	NS	NS	NS
+ control (Glib.)	-	5	175.90±8.50^{ab}	160.30±5.87^{abc}	164.07±5.76^{a}	167.07±4.88^{a}	171.57±4.81^{a}
Normal control (NI)	-	-	183.38±6.10^{ab}	183.88±6.03^{bc}	184.40±6.08^{a}	189.28±4.50^{a}	191.08±4.99^{a}

Values are presented in Mean ± SEM, Groups with similar lowercases are insignificantly different at P < 0.05. * = Single survival
Ns = No survival, NT = Not treated, Glib. = Glibenclamide and NI = Not induced

Table 2. Effect of aqueous and ethanolic leaf and bark extract of *I. gabonensis* on blood glucose level of alloxan-induced diabetic Wistar rats.

Treatment	Solvent	Dose (mg/kg)	Blood Sugar level (mg/dl)				
			0 day (Pre-Induction)	4th day	8th day	12th day	16th day
Leaf	Aqueous	150	53.50±1.94^{b}	463.50±29.32^{b}	280.75±33.98^{b}	146.00±27.30^{a}	103.50±4.50^{ab}
		250	48.75±2.66^{ab}	381.33±39.77^{b}	336.00±37.00^{b}	147.00±27.30^{a}	97.00±2.00^{a}
	Ethanol	150	50.00±1.29^{ab}	562.00±0*	NS	NS	NS
		250	53.50±0.87^{b}	495.00±33.00^{b}	441.00±0*	402.00±0*	385.00±0*
Bark	Aqueous	150	50.75±1.11^{ab}	510.75±39.61^{b}	469.50±28.50^{c}	317.00±0*	268.00±0*
		250	49.75±2.69^{ab}	433.67±31.10^{b}	309.50±16.50^{b}	240.00±34.00^{b}	118.00±9.00^{b}
	Ethanol	150	44.25±1.11^{a}	475.50±18.50^{b}	Hi	498.00±0*	408.00±0*
		250	50.25±1.65^{ab}	416.50±3.50^{b}	Hi	NS	NS
NT	-	-	52.00±1.87^{ab}	503.50±24.50^{b}	NS	NS	NS
+ control (Glib.)	-	5	48.00±2.04^{ab}	468.67±30.82^{b}	249.00±33.41^{b}	98.67±1.76^{a}	86.67±2.73^{a}
NI	-	-	47.75±1.89^{ab}	98.50±3.59^{a}	102.25±1.93^{a}	87.75±3.15^{a}	90.00±2.48^{a}

Values are presented in Mean ±SEM, Groups with similar lowercases are insignificantly different at P < 0.05
* = Single survival, Ns = No survival, NT = Not treated, Glib. = Glibenclamide, NI = Not induced, Hi = above the detection level of instrument (Glucometer)

Haematological parameters assessed in the present study was carried out in order to determine if there will be significant impact of the extracts on the blood parameters since alloxan influence the blood sugar level of animals, moreover two groups with treated ethanolic leaf and bark extracts of 150 and 250 mg/l respectively could not recover from increase in blood sugar level and decrease in the body weight. The assessment of haematological parameters could be used as stated by [5] to reveal the deleterious effect of foreign compounds toxins, chemical and plant extracts on the blood constituents of animals. The study showed that treated diabetic rats with dose of 150 mg/l of both aqueous leaf and ethanolic bark extracts improved the Packed Cell Volume (PCV). Diabetic group administered with leaf aqueous extract at 250mg/l showed an increase in the haemoglobin level (Hb), Red Blood Cell count (RBC) and Platelet level compared to the normal control rats. This may suggest that the extract has a positive impact on the blood parameters which was in agreement with the work done by [5] on n-butanol fraction of *Alchornea cordifolia* leaf extract who reported the improved levels of erythrocyte indices and total protein of the extract. The MCV and MCH

level showed significant decrease in the diabetic groups compared to the normal control group, this could be an indication of presence of macrocytic anaemia i.e. red blood cells (erythrocytes) larger than their normal volume, since increased MCV and MCH values are known to be indicative of macrocytic anaemia [5,23]. However, there was no significant change in the MCHC level in the diabetic groups treated with the extracts or glibenclamide compared to normal control group. These haematological parameters are consistent with the report (24) on the ability of medicinal compounds or drugs in altering the normal range of hematological parameters of animals Table 3.

Table 3. Effect of aqueous and ethanolic leaf and bark extract of *I. gabonensis* on haematology and blood indices of surviving Alloxan-induced diabetic Wistar rats.

Treatment	Leaf		Bark	Positive control (Glib.)	Normal control (NI)
Solvent	Aqueous		Aqueous		
Dose (mg/l)	150	250	250	5mg/kg	
PCV (%)	47.00±0.58^{a}	47.50±0.87^{ab}	47.00±0.58^{a}	50.00±0.58^{b}	46.00 ±0.58^{a}
Hb (g/dl)	15.45±0.55^{a}	16.69±0.23^{b}	15.40±0.12^{a}	17.08±0.07^{b}	15.25±0.03^{a}
RBC (x 10^{6}/ µl)	9.20±0.53^{b}	11.07±0.10^{c}	9.35±0.03^{b}	11.25±0.03^{c}	8.02±0.05^{a}
Platelet (x 10^{3}/µl)	704.50±69.57^{a}	965.00±5.48^{b}	784.00±3.46^{a}	982.00±2.31^{b}	715.00±14.43^{a}
MCV (fl)	51.39±2.37^{c}	42.93±1.16^{a}	49.12±0.20^{bc}	44.45±0.63^{ab}	57.37±1.05^{d}
MCH (pg)	16.87±0.38^{b}	14.98±0.02^{a}	16.93±0.09^{a}	15.80±0.26^{a}	19.02±0.07^{c}
MCHC (g/dl)	32.84±0.76^{a}	33.36±0.06^{a}	33.45±0.27^{a}	34.06±0.19^{a}	33.16±0.48^{a}

Values are presented in Mean ±SEM, Groups with similar lowercases are insignificantly different at P < 0.05

Table 4. Effect of aqueous and ethanolic leaf and bark extract of *I. gabonensis* on white blood cells and differentials of surviving Alloxan-induced diabetic Wistar Rats

Treatment	Leaf		Bark	+ control (Glib.)	- control (NI)
Solvent	Aqueous		Aqueous		
Dose (mg/l)	150	250	250	5mg/kg	
WBC (x 10^{3}/ µl)	12.05±1.01^{a}	15.04±0.03^{b}	12.23±0.07^{a}	12.12±0.11^{a}	10.15±0.32^{a}
Neutrophils	56.50±6.64^{a}	73.00±0.58^{a}	57.50±0.29^{a}	68.00±0.58^{a}	65.00±6.35^{a}
Leucocytes	42.00±6.93^{a}	27.00±0.58^{a}	40.50±0.29^{a}	32.00±0.58^{a}	33.00±6.93^{a}
Monocytes	0.50±0.29^{a}	0.50±0.29^{a}	2.75±0.14^{b}	0.00±0^{a}	2.00±0.58^{b}

Values are presented in Mean ±SEM, Groups with similar lowercases are insignificantly different at P < 0.05

At the dose level of 250 mg/kg of leaf aqueous extract, there was a significant increase in the White Blood Cell (WBC), Neutrophils, and Leucocytes when compared to the normal control group. This may also suggests that leaf extract of the plant may contain some antioxidants that can stimulate stem cells in the bone marrow to produce red blood cells, this therefore is in agreement with some work done of leaf extract that showed improvement in the White Blood Cell, Neutrophils and Leucocytes [5,19,25]. The erythropoietin which is a glycoprotein hormone that stimulates stem cells in the bone marrow enhances rapid synthesis of red blood cell as stated by [19], the increased haemoglobin concentration and red blood cell observed in leaf aqueous extract could suggest that it could contain some active components that can triggers the erythropoietin to stimulates stem cells (Table 4).

4. CONCLUSION

The search for safer drugs to treat diabetes is necessitated by serious adverse effects and cost of the drugs used in current therapeutic practice. Our study highlighted that aqueous extracts of *Irvingia gabonensis* leaf and bark are more antidiabetic than the ethanolic extracts. Further study is necessary to ascertain the toxicity of *Irvingia gabonensis* leaf and bark extracts.

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