

Antidiabetic Activity of Methanol Leaves Extract of *Cymbopogon citratus* and *Heteropogon contortus* in Streptozotocin-Induced Diabetic Albino Rats¹Ali Fredrick Ugadu , ²Orinya O. F., ¹Ominyi M.C., ¹Ebenyi L. N, C., ⁴Nwalo, F. N., ³Ogbanshi M. E., ⁵Ezenwali M. O., and ⁵Nsude C.A¹Biotechnology Department Ebonyi State University Abakaliki ,Nigeria²Medical Biochemistry Department Ebonyi State University Abakaliki, Nigeria³Biochemistry Department Ebonyi State University Abakaliki, Nigeria⁴Department of Biology/microbiology/Biotechnology Alex Ekwueme Federal University Ndufu-Alike IkwoEbonyi State Nigeria⁵Biochemistry Department Enugu State University Of Science and Technology Enugu Nigeria.Corresponding Author: fredrick.ali@ebsu-edu.net; alifredrick13@gmail.com.**ABSTRACT**

Glucose homeostasis is controlled by endocrine pancreatic cells, and any pancreatic disturbance can result in diabetes. Most rural dwellers in Nigeria and other parts of the globe often resort to herbal remedy and dietary control in the treatment of diabetes mellitus (DM). This study was undertaken to investigate the rationale for the use of the leaves of *Cymbopogoncontortus* and *Heteropogoncontortus* as a traditional antidiabetic agent. Methanol extract of both leaves were extracted using standard methods. The extracts were evaluated for antidiabetic effect in Streptozotocin -induced diabetes in albino rats. The blood sugar levels were assayed as indices of diabetes. Forty albino rats weighing 180-280g were used for this study, grouped into four A, B, C and H. Group C and H were further subdivided into C1, C2, C3, C4, H1, H2, H3 and H4 each group made up of four rats. Group A, C and H were exposed with streptozotocin while B was not exposed and served as positive control, group A was the negative control whereas groups C and H were treated with 200, 400, 600 and 800 mg/kg/day of the extract respectively for fourteen days. They were fed with rat feed and water *ad libitum*. The results indicated that intraperitoneal administration of extract (200, 400, 600 and 800 mg/kg/day) exhibited a significant ($P < 0.05$) anti-diabetic effect by ameliorating Streptozotocin -induced increase in blood sugar in a dose -dependent fashion. This study demonstrated that *Cymbopogoncontortus* and *Heteropogoncontortus* have therapeutic effects in diabetes and hence, supporting the traditional uses of these plants by Abakaliki traditional medicine.

Keywords: Diabetes mellitus, *Cymbopogoncontortus*, *Heteropogoncontortus*, Streptozotocin and blood sugar.**INTRODUCTION**

There is a global increase in the prevalence of diabetes mellitus predominantly, related to life styles and the resulting increase in obesity [1]. Diabetes is due to insufficient insulin production or its ineffectiveness. It is a global problem [2]. Diabetes mellitus is a chronic complication of derangement of protein, carbohydrate and fat metabolism arising due to defective insulin secretion or action or both [3].

This disorder of metabolism results in hyperglycemia and glycosuria. The blood sugar level is high over a prolonged period. It develops by regular consumption of sugar sweetened beverages [4] and sedentary lifestyle [5]. Diabetes mellitus is associated with long term complications such as retinopathy, neuropathy and angiopathy [6]. DM is ranked seventh among the leading causes of death in world today and is considered third when its fatal complications are taken into account [7]. Diabetes mellitus (DM) is considered to be one of the most serious endocrine syndromes. It has been estimated that about 171 million people worldwide suffer from diabetes [8], and the use of orthodox drugs in the management of DM has not improved the situation. Plants are well known in traditional medicine for their hypoglycaemic activities [

9]. Available reports indicate that there are more than 800 plants species showing hypoglycaemic activity [10].

There has been increasing demand for the use of plant products with antidiabetic activity due to some reasons which include; low cost, easy availability and lesser side effects. Thus, plants are being continuously explored for their possible effect as hypoglycaemic agents [11].

In Africa, some of the plants commonly used for the treatment of diabetes include, *Brideliaferruginea*, *Dioscoreadumentorum* [12] and *Vernoniaamygdalina* [13]. There are experimental evidence for the hypoglycemic effects of some medicinal plants, at least in experimental model of diabetes [14].

In many countries it is traditional to use plants to control diabetes [1]. Many plants have been used traditionally in Ayurveda and reported to have good antidiabetic activity [16].

*Cymbopogoncitratu*s, (Lemon grass) is a widely used herb in tropical countries, especially in Southeast Asia [17]. The essential oil of the plant is used in aromatherapy, skin rashes etc. The compounds identified in *Cymbopogoncitratu*s are mainly terpenes, alcohols, ketones, aldehyde and esters [18]. Some of the reported

phytoconstituents are essential oils that contain Citral α , Citral β , Nerol Geraniol, Citronellal, Terpinolene, Geranyl acetate, Myrecene and TerpinolMethylheptenone[19]. *C. citratus* also contains reported phytoconstituents such as flavonoids and phenolic compounds, which consist of luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol and apiginin [20].

Heteropogoncontortus is a tropical, perennial tussock grass with a native distribution encompassing Southern Africa, southern Asia, Northern Australia, Oceania, and southwestern North America [21]. The species has also become a naturalised weed in tropical and subtropical regions in the Americas and East Asia [22]. The plant grows to 1.5 metres (4.9 ft) in height and is favoured in most environments by frequent burning [23]. The plants develop characteristic dark seeds with a single long awn at one end

and a sharp spike at the other [24]. The awn becomes twisted when dry and straightens when moistened, and in combination with the spike is capable of drilling the seed into the soil.

The species is known by many common names, including etta (EzamgboEbonyi ,Nigeria) black speargrass, tanglehead, *steekgras* (Afrikaans) and *pili* (Hawaiian). *H. contortus* is a valuable pasture species across much of its range however it has also been responsible for the elimination of the wool industry over much of Australia due to the seeds becoming embedded in the wool and skin of sheep and devaluing the wool and killing the animals [25]. *H. contortus* seeds are also responsible for similar injuries in dogs with thick undercoats, or becoming embedded in the socks and skin of hikers [26].

MATERIALS AND METHODS

Plant Materials

Fresh leaves *Heteropogoncontortus* and *cymbopogoncitratu*s were obtained from Umuoghara in Ezza-North, Ebonyi State

and identified by plant taxonomist Mr Nwankwo Applied Biology Department Ebonyi State University .

Chemicals

The analytical graded chemicals were used for all the experiments. Streptozotocin (sigma chemicals)was used in the experimental protocol .Streptozotocinwas purchased

from sigma chemicals and was dissolved in 0.1M citrate buffer (PH 4.5) at the dose of 60 mg/kg body weight .

Preparation of plant sample

The *Heteropogoncontortus*and*Cymbopogoncitratu*s leaves were washed dried at room temperature for 21 days and were milled thoroughly into powdery form using mechanical blender. The milled *H. contortus* and *C. citratus* were sieved with 2mm sieve 200 gram measured ,dissolved in 400ml of methanol . The system was allowed for 48

hours and further extracted with muslin cloth , the extract were allowed to evaporate and the residuesscrept into sterile bottle and stored in refrigerator till when used .This was used to prepare 200,400,600, and 800 mg/ml concentrations of the extract using normal saline.

Experimental Animals and research design

Forty(40) albino rats of wister strain weighed between 180-280g were used for this study . They were housed in the animal house of Biotechnology department, Ebonyi State University.The animals weregrouped into four (4) as follows: A, B,C and H . Group C and H were sub divided into four groups C1,C2, C3 , C4 H1,H2,H3,and H4 each group

made up of four rats respectively. They were all fed with rat chow dry pellet diet procured from Obum farm 3 Zik avenue Abakaliki Nigeria and water^{ad libitum} , maintained under standard laboratory conditions with dark and light cycle(12:12 hour). The animals were acclimatized for 7days before commencement of this work.

Antidiabetic activity in streptozotocin-induced diabetes model

Diabetes was induced in rats by injecting streptozotocin at the dose of 60 mg/kg body weight, intraperitoneally in to overnight-fasted rats. Diabetes was confirmed by the determination of fasting glucose concentration levels. The rats were then kept for the next 24 h on 10% glucose solution bottles, in their cages to prevent hypoglycemia. After 72 h of injection, fasting blood glucose level was measured. Animals which did not develop more than 300 mg/dL glucose levels, were rejected [27]. The selected diabetic animals were divided in to 3 groups (n=4) and thenormal groupnon-streptozotocin animals was also

added in the study. Group B was kept as normal control (non- streptozotocin rats), and received a single dose of 0.5 mL/100 g of the vehicle, group A was kept as negative control, streptozotocin -induced and received a single dose of 0.5 mL/100 g of the vehicle, group C and H, diabetic-induced were treated with methanol extract, at four dose levels, (200, 400,600,800 mg/kg) treatment was continued for 14 consecutive days oral intubation , at the end of day 14th days, the rats were fasted for 24 hours and blood glucose level were determined.

Preparation of Samples and Glucose Evaluation

At the end of the treatment period, the animals were anaesthetized with chloroform vapour, quickly brought out of the jar and sacrificed. Five milliliter (5ml) of the blood

was collected by cardiac punctureinto sterile EDTA bottle with anticoagulant. The serum was used to assay for blood glucose level.

STATISTICAL ANALYSIS

The data obtained were statistically analyzedusing Analysis of variance (ANOVA) with spss version 2.1.andpresented using tables .

RESULTSTable 1: Effect of methanol extract of *Cymbopogon citratus* on streptozotocin -induced diabetic rats.

Group	Before Induction	After Induction	After Treatment
A (Negative)	70.25±19.81	145±23.51	166.5±16.70
B (Positive)	71.5±14.39	70.5±14.64	70.25±14.66
C1	79.5±5.74	165±23.45	113.25±5.06
C2	72±18.42	151.5±15.15	88±6.48
C3	77.25±11.98	188.75±10.72	75.25±9.91
C4	87.5±17.41	174±19.25	73.5±6.56

Table 2: Effects of methanol extract of *H. contortus* on streptozotocin -induced diabetic rats.

Group	Before Induction	After Induction	After Treatment
A (Negative)	70.25±19.81	145±23.51	166.5±16.70
B (Positive)	71.5±14.39	70.5±14.64	70.25±14.66
H1	83.5±16.90	135.75±13.94	109±9.87
H2	91.25±11.35	151.75±15.78	93.75±5.91
H3	68.5±13.63	178±13.66	82.25±2.22
H4	71.25±20.22	180.75±25.51	72.5±9.33

Table 3: Comparative evaluation of blood glucose level of wistar rats treated with *Cymbopogon citratus* and *H. contortus*

Group	Before Induction	After Induction	After Treatment
A (Negative)	70.25±19.81	145±23.51	166.5±16.70
B (Positive)	71.5±14.3	71.5±14.3	71.5±14.3
C1	79.5±5.74	165±23.45	113.25±5.06
C2	72±18.42	151.5±15.15	88±6.48
C3	77.25±11.98	188.75±10.72	75.25±9.91
C4	87.5±17.41	174±19.25	73.5±6.56
H1	83.5±16.90	135.75±13.94	109±9.87
H2	91.25±11.35	151.75±15.78	93.75±5.91
H3	68.5±13.63	178±13.66	82.25±2.22
H4	71.25±20.22	180.75±25.51	72.5±9.33

DISCUSSION

In general, there is very little scientific knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain active ingredients such as flavonoids, alkaloids, glycosides, terpenoids, etc., which possess antidiabetic effects [28]. Phytochemical analysis of the leaf extract in other reports revealed the presence of flavonoids, alkaloids, glycosides, polyphenols, tannins, saponins, phytosterols and triterpenes in the leaf extract [29].

The present study was undertaken to examine the antidiabetic activity of *Heteropogon contortus* and *Cymbopogon citratus*. Diabetes is a major health problem affecting major populations worldwide. Diabetes mellitus is being increased worldwide. Extensive research is employed to cure

the disease [30]. Blood glucose is an essential parameter in the study of metabolism and diabetes [31]. In this study, exposure of the test animals to STZ selectively destroys the pancreatic insulin β cells, leaving less active cell resulting in a diabetic state. STZ action on β cells is accomplished by characteristic alterations in blood insulin and glucose concentrations. The changes in blood glucose as a result of insulin concentrations reflect abnormalities in β cell functions. STZ impairs glucose oxidation [32], reduction in insulin biosynthesis and secretion. Reports have shown that STZ first abolishes the β cell response to glucose and temporary return of responsiveness which is followed by its permanent loss and cells are damaged [33]. The methanol extract of *Heteropogon contortus* and *Cymbopogon citratus* produced a

significant decrease in the blood glucose level at a dose of 600 mg/kg and 800 kg/kg in diabetic rats. The animals which were treated with of 800 kg/kg of *Heteropogoncontortus* showed a significant decrease blood glucose levels when compared to the 200,400 mg/kg. [Table 1, 2, and 3]. The hypoglycemic effect produced by the extract may be attributed to increased insulin release resembling the mechanism of action of sulphonyl ureas [34].

Administration of methanol extract, for 14 days prevented a significant elevation in glycose thereby increasing normalizing the blood glucose level ($P < 0.05$) in diabetic rats. This could be due to the result of improved glycemic control produced by plant extracts. The plant extracts caused a reduction in blood glucose and may have slowed the degradation of insulin. Reports on plant extract have shown that they improves circulation of blood, regulate microcirculation and empowers the pancrease to optimize insulin production in the body [35]. It also reduces the blood sugar by restoring the capacity of the pancrease for secretion of enough insulin and resumes receptors to insulin cytosine [36].

The changes in blood glucose level provide useful information on the general state of blood after exposure to streptozotocin insult. These alteration in blood glucose of diabetic rat has been corrected in rats treated with methanol extracts of *Heteropogoncontortus* and *Cymbopogoncitratatus* due to the favorable action of phytochemicals present in these plants [37]. *Heteropogoncontortus* and *cymbopogoncitratatus* have antioxidant potential which was confirmed by [38]. The extracts of *Heteropogoncontortus* and *Cymbopogoncitratatus*, have enhanced the restoration of glucose level which were remarkable in the treated group in dose dependent fashion compared to the negative control (Table 1, 2, and 3). Similar results were observed by [39] in Wistar rats intoxicated with paracetamol further treated with propolis, by [40] and in mice intoxicated with lead nitrate and treated with ethanol extract of *Extra coriander* [41]. The effects of *Heteropogoncontortus* and *Cymbopogoncitratatus* extract have reversed the alteration caused by streptozotocin in glucose which was dose dependent.

The antidiabetic action observed for this plant extract is in agreement with the findings of other authors [42]

CONCLUSION

Administration of streptozotocin to albino rats caused damage to pancreatic β cells resulting in hyperglycemia. Treatment of diabetic rats with *Heteropogoncontortus* and

Cymbopogoncitratatus extracts improved glucose levels. This support the use of these plants by traditional herbalist in management of diabetes.

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