ANTI-HYPERGLYCEMIC AND ANTI-OXIDANT ACTIVITIES OF METHANOL EXTRACT OF *GONGRONEMA LATIFOLIUM*

ABSTRACT-

Gongronema latifolium (Asclepiadiaceae) is used as bitterspice or flavouring agent in many traditional Nigerian dishes. It is well known for medicinal and nutritional purposes like anti-hyperglycemic, anti-oxidant, antitussive and widely distributed in the southeastern states of Nigeria. Objective of present study was to evaluate the anti-hyperglycemic and anti-oxidant effect of the methanol extract of *Gongronema latifolium* leaves.

The methanol extract of G. latifolium at doses of 250 and 500 mg/kg were studied for anti-hyperglycemic effect in alloxan-induced hyperglycemic rats. The variation in blood glucose level in normal and experimental rats on 0, 7, 14 and 21 days of treatment has also been recorded. Treatment with G. latifolium extract showed signs of recovery as comparable with the standard drug glibenclamide (0.25 mg/kg). The effect of different treatment on body weight of rats was also determined.

The antioxidant properties of the extracts as determined by the DPPH free radical scavenging assay. The result showed that *G. latifolium* extract caused a concentration dependent percentage increase of antioxidant activity.

Study concludes that methanol leaf extract of *G. latifolium* possessessignificant anti-hyperglycemic and anti-oxidant activities.

Keywords: *Gongronema latifolium*, anti-hyperglycemic, anti-oxidant, carrageenan-induced paw edema.

INTRODUCTION

Plants are extremely an important source of medicinal agents. Sinceancient times, plants have been used due to their impactful pharmacological properties. WHO estimates that 80% of the population living in rural areas is dependent on herbal medicine for their health needs. Crude plant extracts in the form of infusion, decoction, tincture or herbal extracts have been traditionally used by the population for the treatment of various diseases[1]. Plant used in treatment of diseases is as old as civilization and traditional medicines are still a major part of habitual treatments of different maladies[2].

Diabetes mellitus is the commonest endocrine disorder that affects more than 171 million people worldwide. It is a condition in which a person has a high blood sugar level. The reason may be either the body doesn't produce enough insulin, or because body cells don't properly respond to the insulin that is produced[3]. When the blood glucose increases for example, after eating food material, insulin is released from the pancreas to normalize the glucoselevel. If the body cells do not absorb glucose, the glucose accumulates in the blood, leading to vascular, neuron, and other complications[4].

Our body is exposed to a variety of oxidizing agents. Oxidation is a chemical reaction that produces free radicals, leading to chain reactions that may damage cells[5]. Body is equally inbuilt with antioxidants to cater for the free radicals generated from the oxidants, so a balance between the production of free radicals and neutralization by antioxidants is remains maintained. Antioxidants are substances known to protect the body from damagecaused by reactive oxygen

species induced oxidative stress[6]. Oxidative stress results because of imbalance between formation and neutralization of free radicals by antioxidants, it results to oxidative stress. Oxidative stress has been implicated in the etiology of diseases such as cardiovascular diseases and lung cancer.

Antioxidants from natural sources have a higher bioavailability and therefore higher protective efficacy comparison of synthetic antioxidants[7].

Gongronema latifolium (Asclepiadiaceae) is used for medicinal and nutritional purposes widely distributed in the southeastern states of Nigeria[8]. Apart from beingused as bitter spice or flavouring agent in many traditional Nigerian dishes the plant leaves has been found very efficacious as an antidiarrhoeal and antitussive. Aerial parts are taken to treat cough, intestinal worms, dysentery, dyspepsia and malaria[9]. It is also taken as a tonic to treat loss of appetite. A decoction of leaves or leafy stems is commonly taken to treat diabetes and high blood pressure[10].

The objective of the present study was to evaluate the anti-hyperglycemic and anti-inflammatory properties of methanol leaf extract of *Gongronema latifolium.*

MATERIAL AND METHODS

Collection and preparation of leaf samples:

Fresh leaves and roots of *G. latifolium* were collected from Niger Delta and authenticated.

Extraction of plant materials

Fresh leaves of *G. latifolium* were washed and allowed to dry air at roomtemperature (28 to 30^{O}C) for two weeks, to avoid the escape of volatile components by oven-drying. The dried leaves were milled into fine powder using the Christy-Norris hammer mill and passed through a1mm sieve to obtain a fine powder. 2 kg of the sample was percolated in methanol for 48 hrs; this was then filtered through a Whatman filter paper No.1. The filtrate was evaporated to dryness on a hot plate at an initial temperature of 100^{0}C and the dry powder obtained was suspended in 10ml distilled water, stirred and refiltered[11].

Assessment of hypoglycaemic activity

The approval of the Institutional Animal Ethics Committee was obtained before starting the study. An international protocol for conducting experiments on animals were followed. Healthy wistar rats of eithersex having weight 150 - 200 g were selected for this activity[12]. They were housed in standard condition of temperature (25 ± 2 ^{0}C) with 12 h light per day cycle. Before induction of diabetes weigh and normal glucose levels of rats were determined and recorded as Day 0. The acclimatized animals were fasted for 24 h with water ad libitum[13].

Hyperglycemia was induced using a single intraperitoneal injection of alloxan monohydrate (160 mg/kg).

All animals were returned to their cages and given free access to food and water. Blood glucose levels were monitored by using a Glucometer after 72 h of injectionand recorded as 1st day.

Rats with fasting blood glucose >7.8 mmol/l or 140 mg/dl were considered hyperglycemic and were selected for the study.

Diabetic rats were randomly assigned to 5 groups, each group contains six animals.

The animals were grouped as follow

- Group I: Normal control
- Group II: Diabetic control
- Group III: Diabetic rats treated with *G. latifolium* extract (250 mg/kg)
- Group IV: Diabetic rats treated with *G. latifolium* extract (500 mg/kg)
- Group V: Diabetic rats treated with glibenclamide (0.25 mg/kg).

Blood samples were obtained from the cut tail tip of the conscious rat and glucose test strip soaked with blood and then inserted to be read by the glucometer. Blood glucose levels were examined after 2, 12, 24, 72 hrs of orally administration of test drugs[14].

Table 1: The effect of different treatment on fasting blood glucose levels in alloxan induced diabetic rats

Treatment	Blood glucose level (mg/dl)			
	0th Day	**7th Day**	**14th Day**	**21st Day**
Normal control	90.4± 0.25	93.21± 0.26	92.47± 0.22	91.65± 0.15
Diabetic control	247.58± 0.43	265.28± 0.31	280.37± 0.41	300.48± 0.42
G. latifolium **(**250 mg/kg)	245.72± 0.51	200.82± 0.42	150.47± 0.53	122.37± 0.37
G. latifolium (500 mg/kg)	247.38± 0.09	195.28± 0.51	142.37± 0.08	120.25± 0.22
Glibenclamide (0.25 mg/kg)	248.46± 0.13	179.23± 0.38	125.38± 0.06	112.37± 0.07

N = 6, $p < 0.05$

Table 2: The effect of different treatment on body weight of alloxan induced diabetic rats

Treatment	Change in body weight (gm)		
	Initial	**10th Day**	**21th Day**
Normal control	154 ± 0.31	174.00 ± 0.8	180.50 ± 0.32
Diabetic control	181 ± 0.28	165.00 ± 0.64	140.67 ± 0.43
G. latifolium **(**250 mg/kg)	158 ± 0.17	147.67 ± 2.10	148.33 ± 0.7
G. latifolium (500 mg/kg)	174 ± 0.36	149.00 ± 0.7	144 ± 1.2
Glibenclamide (0.25 mg/kg)	176 ± 0.28	157.00 ± 0.53	167.83 ± 0.9

N = 6, $p < 0.05$

Anti oxidant study

DPPH antioxidant assay[14]

The free radical scavenging activity of *G. latifolium* extract was analyzed by, 1, 1-diphenyl-2-hydrazyl (DPPH) photometric assay. 2 ml of test extract at concentrations ranging from 10 to 400 µg/ ml was each mixed with 1 ml of 0.5 mM DPPH in methanol.Absorbance at 520 nm was taken after 30 min incubation in the dark at room temperature using a spectrophotometer[15]. The experiment was done in triplicates and the percentage antioxidant activity was calculated as follows:

% of DPPH free radical scavenging= (Absorbance of (control-blank))/(Absorbance of control) x 100

Where 1 ml methanol and 2 ml extract were used as blank, while 1 ml 0.5 m M DPPH solution and 2 ml methanol was used as control. Ascorbicacid was used as reference standard

Table 3: Antioxidant activity of the methanol extract of G. *latifolium*

Sample	Conc (µg/ml)	Absorbance	Absorbance of control	Mean % DPPH scavenging activity
Ascorbic acid	10	0.069	0.812	86.43 ± 0.17
	100	0.060		90.63± 0.32
	200	0.049		89.49± 0.53
	400	0.038		93.69± 0.82
Methanol extract of *G. latifolium*	10	0.269		70.33± 0.38
	100	0.250		76.09± 0.24
	200	0.215		78.18± 0.31
	400	0.190		80.27± 0.78

N=3, p<0.05

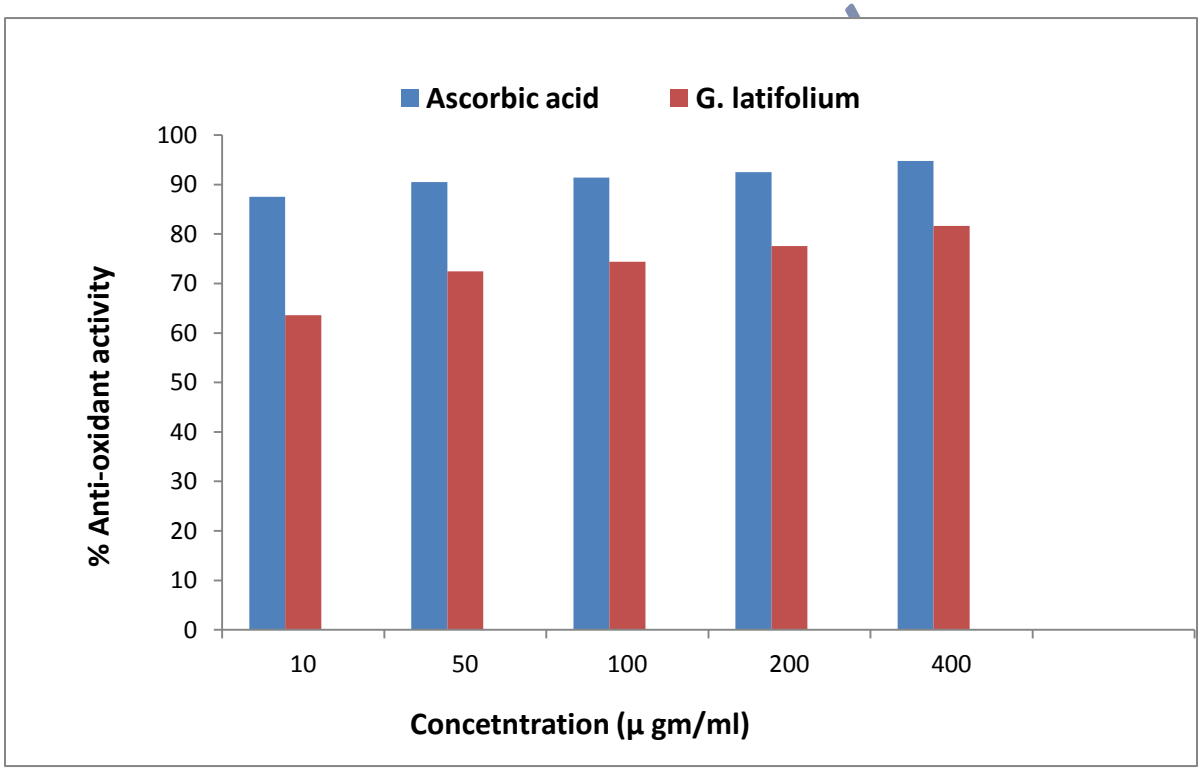

Figure 1: Percentage antioxidant activity of *G. latifolium* extract using DPPH photometric assay.

RESULTS AND DISCUSSION

The anti-hyperglycemic effect of methanol extract of *G. latifolium* leaves was evaluated for 21 days. Alloxan-induced hyperglycemia is due to selective toxicity of alloxan on thepancreatic beta cells, generation of superoxide radicals and cytotoxicaction mediated by generation of reactive oxygen species. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells[16].

The effect of administration of extracts of G. latifolium on blood glucose level of diabetic rats is shown in table 1. Treatment of extract of G. latifolium showed a significant reduction in the blood glucose level.

It also shows that 0.25 mg/kg glibenclamide is lowering glucoselevel significantly compared to normal control. Leaf extract at a dose of 500 mg/kg was more effective in reducing the blood glucose level than a dose of 250 mg/kg. The variation in blood glucose level in normal and experimental rats on 0, 7, 14 and 21 days of treatment has also been recorded.Treatment with G. *latifolium* extract showed signs of recovery as comparable with the standard drug glibenclamide.

There was significant loss in body weight of diabetic rats compared to normal rats. In diabetics, glucose is not available therefore the cells use alternatively proteins for energy; consequently due to excessive breakdown of tissue protein a loss in body weight occurs. Body weight slightly increased in the normal control rats compared to initial bodyweight, whereas in diabetic control rats there was a significant decrease in body weight. Groups that were treated with glibenclamide and G. latifolium extract (250 and 500 mg/kg) showed significant reduction in bodyweights. The final body weights of treated groups were significantly lower than the final weights of normal control group. Hence, present study showed a good antidiabetic response of leafextract against the experimental animals.

The antioxidant activity was done in vitro using 1,1-diphenyl-2-hydrazyl (DPPH) spectrophotometric assays. Figure 1 shows the antioxidant activity of *G. latifolium* and ascorbic acid standard. The result showed that *G. latifolium* extract caused a concentration dependent percentage increase of antioxidant activity. DPPH is a stable free radical, it accepts an electron or hydrogen radical to become a stable diamagnetic molecule which iswidely used to investigate radical scavenging activity. The degree of discoloration indicates the radical-scavenging potential of the antioxidant. Result shows that DPPHscavengingwas increased in a concentration dependent manner compared to ascorbic acid[16].

Statistical Analysis

Graph Pad Prism Version 5.0 for Windows was used for all statistical analyses. Data are presented as mean ± SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparisontest.

CONCLUSION

At present scenario, many researchers are showing their interest in medicinal plants for routine scientific investigation of numerous plants extract for biological effects and potential therapeutic properties in human. Methanol leaf extract of *G. latifolium* haveanti-hyperglycemic and anti-oxidant activities. The methanol extract have antihyperglycemic activity in diabetic rats, most likely to be associated with glucose uptake increasing mechanism. Lower doses of the extract should be tried in future study to establish the most appropriate dose for clinical trial. *G. latifolium* leabes has shown to be a potential anti-diabetic and thus it can be a promising source for anti-diabetic agent.In the present study, the observed DPPH scavenging activity of the methanolic extract of *G. latifolium* might be useful for the development of newer and more potent natural antioxidants.

So, present study concluded that detailed investigation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drug for many dreaded diseases includingdiabetes mellitus.

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