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Research Article

Inhibitory Intestinal Glucose Uptake and Antioxidant In-Vitro Potentials of Aqueous Leaf Extract of *Leptadenia hastata* (Pers)

Kabir N[1*], Umar A I [2], Atiku M K[3], Usman M M[4], Yunusa I[5]

[1]*Department of Biochemistry, Federal University Dutse, Dutse, Jigawa State, Nigeria,*
[2]*Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria,*
[3]*Department of Biochemistry, Bayero University Kano, Kano State, Nigeria,*
[4]*Department of Chemical Pathology and Immunology, Aminu Kano Teaching Hospital, Kano State, Nigeria,*
[5]*Department of Biochemistry, Kano State University of Science and Technology, Kano State, Nigeria*

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ABSTRACT

Use of potent antioxidants and inhibitors of intestinal glucose uptake are one of the current therapeutic approaches employed clinically to control post porandial hyperglycemia and hyperglycemic induced oxidative stress for achievement of good glycemic control and to prevent the onset of diabetic complications. The present study was aimed at evaluating the in-vitro antioxidant and inhibitory intestinal glucose uptake potentials of the aqueous leaf extract of *Leptadenia hastata* (LH) for achievement of good glycemic control. The aqueous leaf extracts of LH was assessed using standard methods by studying the ferric reducing and DPPH radical scavenging activities using ascorbic acid as a standard drug and alpha amylase and alpha glucosidase inhibitory activities using acarbose as standard drug for the assays. Results showed that the aqueous leaf extract of LH possessed a moderately low free radical reducing power (IC_{50} 1200 µg/ml) and a high radical scavenging activity (IC_{50} 651 µg/ml respectively. In addition, the extract possessed inhibitory activities for alpha amylase (IC_{50} 1.00 µg/ml) and alpha glucosidase (IC_{50} 2.62 µg/ml). The presence of alkaloids (3.88±1.71mg/g), saponins (18.92±1.66mg/g), phenols (20.96±4.81mg/g) and tannins (2.1±1.23mg/g) in the aqueous leaf extract of LH might be responsible for the pharmacological activity of LH. Conclusively, aqueous leaf extract of LH has a potential for use in the control of post porandial hyperglycemia and diabetes induced oxidative stress and can be used for future in-vivo antidiabetic studies.

Keywords: Glucose intake, *Leptadenia hastata*

INTRODUCTION

According to the International Diabetes Federation's update, diabetes was estimated to affect 415 million adults with a projected increase of 642 million by 2040 with the increase projected to occur in low and middle income countries[1]. The increase of diabetes in developing countries follows the trend of urbanization and life style changes, perhaps as a result of "western style" diet[2]. Diabetes mellitus (DM) commonly referred to as diabetes is a metabolic disorder characterized by alteration of carbohydrate, lipid and protein metabolism[3]. Hyperglycemia is the clinical hallmark of diabetes but the etiology of this heterogeneous disorder likely involves multiple genetic and environmental interaction that ultimately result in alteration in insulin secretion, insulin action or both[4]. Long term effects of hyperglycemia leads to development of microvascular and macrovascular complications[5].

Hyperglycemia is usually accompanied by increased generation of reactive oxygen species or impaired antioxidant defenses which ultimately progress to development of oxidative stress[6]. The detrimental effects of hyperglycemic induced oxidative stress through increased glucose flux through polyol pathway, formation of advanced glycated end products and an increased generation of superoxide radical from the respiratory chain have been observed to be one of the major risk factor for macrovascular complications of diabetes hence the need to achieve a good glycemic control[7]. Previous studies on diabetes have identified control of postporandial blood glucose as one of therapeutic strategy towards achievement of good glycemic control. This is achieved by use of inhibitors of hydrolysis of starch; the major dietary carbohydrates such as alpha glucosidase and alpha amylase[8]. Of recent, many diabetic patients have opted for traditional herbal medicines alone or in conjunction with conventional hypoglycemic agents and plants as a result of their adverse effects, cost and non availability. In Northern Nigeria over hundred species of plants are used for management of diabetes.

Leptadenia hastata Decne (Asclepiadaceae) is a widely distributed African herb commonly used as a vegetable with vast nutritive and therapeutic properties. It has been considered beneficial against milk drying, cough, sexual impotency[9], trypanosomiasis[10], acute rhinopharygitis,

**Author for Correspondence*

Table 1: Phytochemical Screening and concentrations in the Leaves of LH

Chemical Constituents	Test	Aqueous extract of lh	Concentration (mg/g)
Alkaloids	Mayer's Test Dragendroff's Test Wagner's Test	+	3.88±1.71
Glycosides	Borntrager's Test Fehling's Test	-	-
Anthraquinones	Borntrager's Test	-	-
Saponins	Frothing Test	+	18.92±1.66
Phenols	Pyridins-Ferric Chloride Test	+	20.96±4.81
Terpenoids	Libermann-Burchard Test	-	-
Flavonoids	Sodium hydroxide Test	-	-
Resins	Acetic anhydride test	-	-
Balsams	Ferric chloride test	+	-
Tannins	Ferric Chloride Test Potassium dichromate Test	+	2.10±1.23
Cardiac glycosides	Kelle Killiam test	-	-
Steroids	Libermann-Burchard Test Sulphuric acid Test	-	-
Phlobatannins	Hydrochloric acid test	-	-
Carbohydrates	Molisch Test	-	-

(+) = Present (-) = Absent LH- *Lepetadenia hastata*

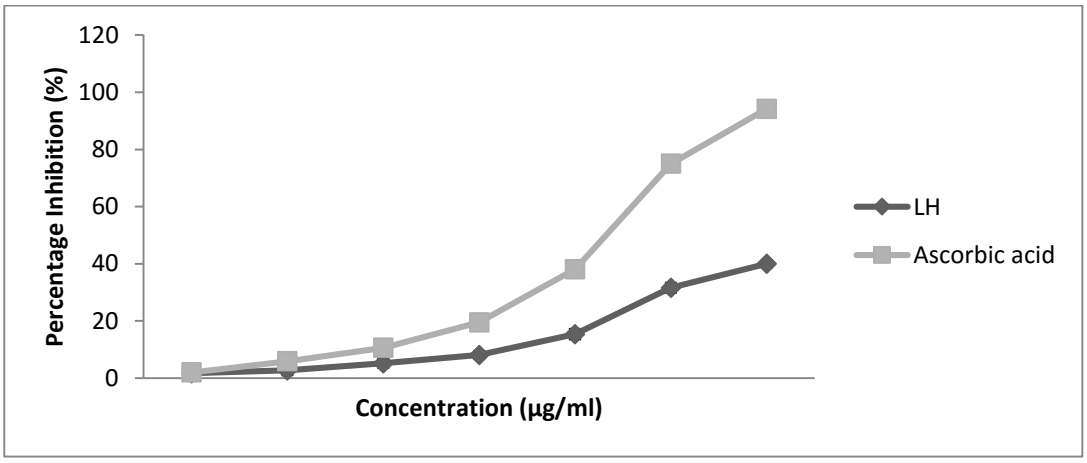

Figure 1: Ferric Reducing Antioxidant Activity of Aqueous Leaf Extracts of LH and Ascorbic acid Mean±SD of 3 replicates

hypertension and wound in human being[11]. Traditionally, the cooked leaves are eaten with ground groundnut cakes and are a common delicacy of northern Nigeria as such can be considered safe for use. The dried powdered leaves are sprinkled on food or pap or in aqueous decoction for different medicinal purposes. Traditional herbal healers use the leaves of LH as common ingredients in anti diabetic poly herbal formulations used by traditional herbal healer. But despite its wide spread use, its use for management of diabetes still lacks scientific backing hence the need for research to understand its possible mechanism of action as a basis for drug development alone or its use in synergy with other traditional plants.

In view of the above, the present study was aimed to evaluate the in-vitro antioxidant and inhibitory glucose uptake potentials of aqueous leaf extract of LH for achievement of good glycemic control. This would provide leads for studying the in-vivo antioxidant and antidiabetic potentials and for use in the control of postporandial hyperglycemia and diabetic induced oxidative stress thereby delaying the onset of diabetic complications.

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of LH were collected from natural vegetation in March 2013 and were washed to remove undesirable material and sand. Voucher specimens (ABU900220) were deposited in the herbarium of Biological Sciences Department, Ahmadu Bello University Zaria. The leaves were dried under shade until dry and the dried leaves were ground and passed through a meshed sieve. The powered leave (500g) was soaked with 2000 milliliters of water and allowed to stay at room temperature for 48 hours. The mixture is then filtered through a muslin cloth then through Whatman filter paper no 1. The resulting filtrate was concentrated to dryness in a water bath set at 60^0C. The

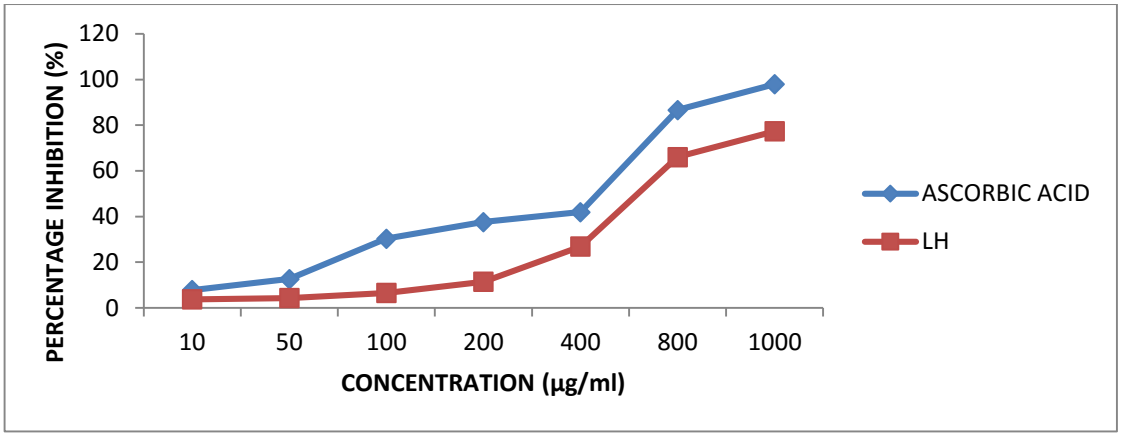

Figure 2: DPPH Radical Scavenging Activities of Aqueous Leaf Extracts of *Leptadenia hastata* and Ascorbic acid Mean±SD of 3 replicates

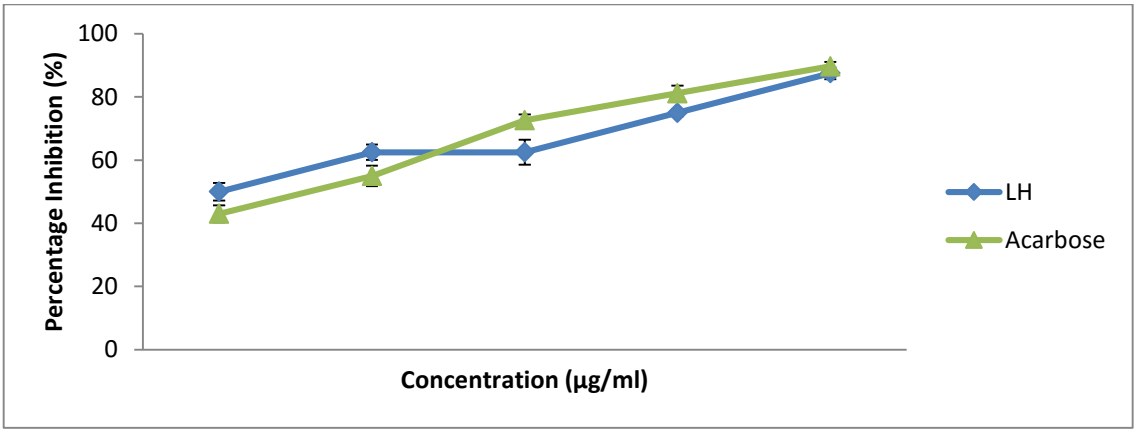

Figure 3: Alpha Amylase Inhibitory Activities of LH and Acarbose Mean±SD of 3 replicates

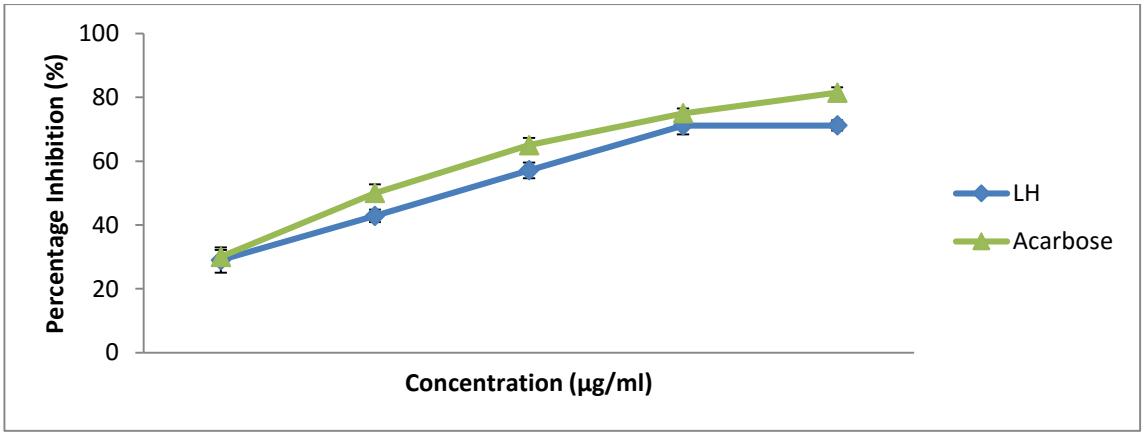

Figure 4: Alpha Glucosidase Inhibitory Activities of LH and Acarbose

extract yield was calculated using the equation: $\frac{\text{Weight of dry extract}}{\text{weight of starting material}}$ X 100

Preliminary phytochemical screening and quantification

The preliminary phytochemical screening of the extract was carried out using standard methods as described by Sofowora[12] and Trease and Evans[13].

Determination of Radical Scavenging Activity using I,1-diphenyl-2-picrylhydrazyl (DPPH)

The ability of the aqueous extract to scavenge DPPH free radical was assessed as described by Shen *et al.*,[14] with some modifications. Varying concentrations of plant and/or standard (10-1000 µg/ml) in methanol and 0.3 mM DPPH in methanol was added. The mixture was vigorously shaken and allowed to stand at room temperature in the dark for 30 minutes. The change in color from deep purple to light yellow was measured at 517nm. Ascorbic acid was used as a positive control for the assay. The assay was carried out in triplicates. The decrease in absorbance was then converted to percentage inhibition using the following equation:

Table 2: IC_{50} Values (µg/ml) For Various Activities of the Aqueous Leaf Extracts of LH, Ascorbic acid and Acarbose

Activity	LH (µg/ml)	Ascorbic acid (µg/ml)	Acarbose (µg/ml)
Ferric Reducing Power	1200	531	--
DPPH Scavenging Activity	651.28	425.29	--
Alpha Amylase Inhibition	1.00	--	1.47
Alpha Glucosidase Inhibition	2.62	--	2.20

$$\%\ Inhibition = \frac{\text{Abs517 (Control)} - \text{Abs517(sample)}}{\text{Abs517 (control)}}\ X\ 100$$

The concentration that causes 50% inhibition (IC_{50}) was determined from plots of percentage inhibition versus concentration.

Determination of reducing power activity

The reducing power ability of the aqueous extract of LH to was evaluated as described by Kumar and Hemalatha[15] with some modifications. Aliquots of varying extract and/or standard (10-1000 µg/ml) concentrations were mixed with 0.2 M phosphate buffer (pH 6.6) and 1% ferricyanide and incubated at 50^0C for 20 minutes. 10 % TCA was added to the mixture and centrifuged ate 980 X g for 10 minutes. The supernatant was mixed with ferric chloride and the absorbance of the solution was measured at 700 nm using a spectrophotometer. Ascorbic acid was used as a positive control.

Alpha amylase inhibitory activity assay

The alpha amylase inhibition assay was carried out as described by McCue and Shetty[16]. Rat pancreas homogenate was used as a crude enzyme source. Assay mixture containing enzyme, and various plant extract/standard (10-100ug/ml) concentration were incubated for 10 minutes at room temperature which was followed by addition of 1% starch (0.006M Sodium Chloride, 0.02M sodium phosphate (pH 6.9) and Starch). The reaction was then terminated by the addition of 3,5-dinitrosallycylic acid and absorbance was measured at 540 nm. Acarbose was used as a positive control in this assay. Samples were assayed in triplicates. The results were expressed as percentage inhibition calculated as follows:

$$\%\ Inhibition = \frac{\text{Abs540 (Control)} - \text{Abs540 (sample)}}{\text{Abs540 (control)}}\ X\ 100$$

IC_{50} value was obtained from linear regression plot of percentage inhibition against concentration.

Alpha glucosidase inhibitory assay

The alpha glucosidase activity was determined as described by Kim et al[17] using p-nitorphenyl-α-D glucopyranoside as substrate. Rat small intestine homogenate was used as crude enzyme source. Varying concentration range of 10-100 µg/ml was used. Acarbose was used as a positive control for this assay. The percentage inhibition was calculated using the following formula:

$$\%\ Inhibition = \frac{\text{Abs405 (Control)} - \text{Abs405 (sample)}}{\text{Abs405 (control)}}\ X\ 100$$

IC_{50} value was obtained from linear regression plot of percentage inhibition against concentration.

Statistical analysis

Results were expressed as mean ± standard deviation.

RESULTS

The extraction yield was 27.14% (w/w) dry matter. To preliminary phytochemical screening revealed the presence of alkaloids, phenols, saponins, Tannins and balsams as presented in (Table 1).

Figure 1 presents the result of ferric reducing activity of varying concentrations of the aqueous leaf extract of LH and standard ascorbic acid. The aqueous leaf extract of LH revealed a moderate dose dependent ferric reducing activity with a maximum activity (44.0±0.25) when compared to ascorbic acid (94.0±0.66) at 1000µg/ml. The DPPH radical scavenging activities at various dose concentrations (10-1000 µg/ml) of aqueous extracts of LH and ascorbic acid are presented in Figure 2. A dose dependent activity of the aqueous extract of LH and ascorbic acid were observed.

The alpha amylase and alpha glucosidase inhibitory activites of various concentrations (10-100 µg/ml) of aqueous extract of LH and acarbose are presented in Figure 3 and Figure 4. The maximum inhibitory activity for alpha amylase and alpha glucosidase of the aqueous leaf extracts of LH and acarbose was observed at the highest extract concentrations (100 µg/ml) with IC50 values of 1.00 µg/ml and 1.47 µg/ml and 2.62 µg/ml and 2.20 µg/ml respectively. A better alpha amylase inhibitory activity was displayed by the aqueous extract of LH than the positive control acarbose while on the other hand, acarbose displayed a higher alpha glucosidase inhibitory activity than the aqueous leaf extract of LH.

The IC_{50} for the antioxidant and hypoglycemic activities of aqueous extract of LH are presented in Table 2. For ferric reducing power activity of the aqueous extract of LH an IC50 value of >1000 µg/ml in comparison with positive control ascorbic acid of 531 µg/ml depicting a low reducing activity was observed. The 50% inhibitory activity (IC50) of the aqueous leaf extract of LH and ascorbic acid were 651.28 µg/ml and 425.29 µg/ml respectively for DPPH scavenging assay. This indicated a high antioxidant activity of the extract. The IC_{50} activities of LH and acarbose for the alpha amylase and alpha glucosidase inhibitory assay was found to be 1.00 µg/ml, 1.47 µg/ml and 2.62 µg/ml, 2.20 µg/ml respectively.

DISCUSSION

The presence of phenols, saponins, alkaloids and tannins in the aqueous leaf extract suggests correlates with a review by Thomas et al[18]. The vast majority of medicinal plants are observed to exert their antioxidant and antidiabetic activities due to the presence their phytochemicals. Phenolic compounds are secondary metabolites found in many plants suggested to be antioxidants with redox potentials. They are reported to exert their antioxidant activities through antioxidant

enzymes activation, ability to remove free radicals and chelate metal ions as well as act as reducing agents, hydrogen donators and inhibitors of oxidases[19,20]. As such the presence of phenols in the aqueous extract of LH was indicative of the extract's ability to act as reducing agent and a radical scavenger.

Normally production of free radicals is balanced by the antioxidant defence system. Ferric reducing power and DPPH radical scavenging in vitro assays are commonly used to evaluate antioxidant activities of natural product from plant and microbial sources[21]. DPPH is used to measure radical scavenging potentials of substances capable of donating a hydrogen atom. The ability of the aqueous extract of LH to reduce Ferric chloride and scavenge DPPH translates into a strong antioxidant potential of the extract and its thus could be capable of protecting biological tissues and membranes from the deleterious effects of free radicals. This agrees with findings of a study of Thomas et al.[18] where they observed that roots and stems of LH possessed antioxidant activity.

Inhibition of intestinal glucose uptake by inhibiting major carbohydrate hydrolyzing enzymes alpha amylase and alpha glucosidase have been shown to be the major hypoglycemic therapeutic approach to prevent post porandial hypoglycenmia[22].

Post porandial hypoglycemia is an early derangements in diabetes that prevents the achievement of good glycemic control which leads to progression of diabetic complications[23]. Alpha amylase and alpha glucosidase activity of the aqueous extact of LH was observed in the present study. Rohn et al.[24] have attributed the alpha amylase and alpha glucosidase activity of some antidiabetic plants to their phytochemical phenolic contents of the extracts which are observed to interact with and/or inhibit the protein enzyme. Findings of the present study correlates with the study of Bello et al.[25] where they also reported an alpha glucosidase activity in some plant parts of LH extracts. However, Brinker et al.[26] have attributed the hypoglycemic activity of some species of plant to their alkaloid phytochemical content. This agrees with the findings of present study where the aqueous leaf extract of LH revealed the presence of alkaloids and hence the ability to inhibit carbohydrate hydrolyzing enzymes; alpha amylase and alpha glucosidase. The in-vitro hypoglycemic potential of the aqueous extract of LH is an indication that the plant could have potent in-vivo hypoglycemic activity.

CONCLUSION

Aqueous leaf extract of LH could be used to ameliorate hyperglycemia and hyperglycemia induced-.oxidative stress as evidenced by its ability to inhibit intestinal glucose uptake via inhibition of alpha amylase and alpha glucosidase and reduce and scavenging free radicals in-vitro. This could provide basis for development of drug to control post porandial hyperglycemia and oxidative stress to achieve good glycemic control. The present results also provides leads for further in-vivo study of the aqueous extract of LH in chemically-induced animal models alone or in combination with other plants as is commonly practiced by traditional herbal healers. In addition, isolation and evaluation of antidiabetic effect of the active principles of the aqueous leaf extracts of LH would be the next line of study.

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