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Phytochemical Screening and Anti-diabetic Potential of Ethanol Extracts of Cooked *Allium cepa* L. (onion)

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Abstract The acclaimed traditional use of vegetables as antidiabetic remedies has now been considered as one of the two basic diabetes mellitus control factors: food and medication. Allium species such as *Allium cepa* (onion, Hausa:Albasa) has attracted particular attention of modern medicine because of its widespread health use. In the present study the antidiabetic (hyperglycemic) potential of ethanol extract of cooked, dried and pulverized *Allium Cepa* bulbs was assessed in alloxan-induced diabetic mice. Hyperglycemia was induced by the injection of 150 mg/kg (*i.p.*) of alloxan monohydrate freshly dissolved in physiological saline. Doses (150, 450 and 750 mg/kg) per os, of the extracts were separately administered to a group of five diabetic mice in the study. The activity was compared with reference standard glibenclamide (2 mg/kg, p.o.) and negative control of physiological saline. Treatment of the alloxan-induced diabetic mice with the ethanol extracts of *Allium cepa* decreased the raised blood glucose levels significantly ($P< 0.05$) in a dose-dependent manner. Results of phytochemical screening of the ethanol extracts of cooked *Allium cepa* bulb have indicated the presence of cardiac glycoside, steroids, terpenoids, alkaloids, flavonoids, and anthraquinones. Moreover, some of the phytochemicals observed in this plant extract have previously been observed to contribute to hyperglycemic effects.

Keywords Alloxan monohydrate,uncooked *Allium cepa*, diabetes mellitus, physiological saline, glibenclamide, albino mice

Introduction

Among the modern chronic diseases in the world today, diabetes mellitus has been reported to be number one killer [1] and as also reported elsewhere 60% of the world's diabetic patients are found in the Asian countries [2]. In the United States, about 8.3% of the total population have diabetes and an additional 79 million adults have pre-diabetes [3], a condition where the blood glucose levels are higher than normal but not high enough to be diagnosed as diabetes (i.e., fasting blood glucose ≥100 mg/dL and ≤ 126 mg/dL). A recent modeling study estimated that 52% of the population in the United States will have diabetes or pre-diabetes by 2020 [4]. Diabetes mellitus (DM) is a complex metabolic disorder that results from defects in insulin secretion, action, or a combination of both [5]. It is a disease that affects the metabolism of carbohydrate, protein and fat. There are two major types of diabetes mellitus disease [6]. Type 1 diabetes mellitus is caused by autoimmune-mediated destruction of pancreatic islet β-cells resulting in insulin deficiency. The type 2 diabetes mellitus, on the other hand, is as a result of either an inability of the islet β-cells to secrete sufficient insulin or reduced insulin activity or a combination of both. Essentially, diabetes mellitus renders the body cells unable to store glucose [7-8]. Type 1 diabetes mellitus is usually treated by the use of injected insulin whereas for the treatment of type 2 diabetes mellitus many oral hypoglycemic agents are used [9].

Orthodox treatment of diabetes mellitus includes modification of lifestyle, such as diet and exercise and the use of insulin or oral hypoglycemic drugs [10]. The four important classes of oral hypoglycemic drugs are sulphonylureas, biguanides, thiazolidinediones, and α-glucosidase inhibitor. In addition, some of the more recently approved drugs such as glucagon-like peptide-1 agonists, dipeptidyl peptidase-IV inhibitors, and amylin analogues are also used in the management of diabetes [9]. These conventional anti-diabetic drugs have been designed to most importantly slow down glucose absorption from the gut, secretion of enough insulin by β-cells, or increase insulin sensitivity at target tissues. Use of insulin or oral hypoglycemic agents is associated with drawbacks such as ineffectiveness on oral administration, short shelf life, requirement of constant refrigeration and in the event of excess dosage fatal hypoglycemia [10]. Due to adverse side effects include hypoglycemia and hepatic dysfunction there have been persistent efforts to screen potential compounds that can "cure" diabetes mellitus, for example by stimulating β-cell regeneration and preventing apoptosis, leading to a return of endogenous control of glucose homeostasis [6]. Naturally occurring plant compounds are attractive candidates because they are abundant in nature, inexpensive to produce and may have fewer side-effects than the currently used pharmaceutical compounds.

Besides adding a delicious taste and flavour to food, onion (*Allium cepa*) serves as a good traditional herbal medicine. The plant is a bulbous herb that belongs to the vegetable family Alliceae. The pungent taste of onion is due to volatile oil allyl-propyl-disulphide present in it. Onion is the most commonly used vegetable in the world food preparations especially in the tropical countries. It is a very commonly used vegetable, ranks third in the world production of major vegetables [11]. Intake of onion is reported to have many health benefits that include the prevention of hypoglycemia [12-13] hepatic dysfunction [14] and hypertension [15], as well as improving diabetic condition [16-17]. Studies on the ability of onion to treat the chemically induced non-insulin-dependent diabetes have reported hypoglycemic effect in animals [18-19]. In the present study we have investigated the effect of ethanol extract of cooked onion bulb on blood glucose levels in alloxan monohydrate - induced diabetic albino mice compared to glibenclamide as a reference standard. Alloxan monohydrate destroys β-cells of Islets of Langerhans of the pancreas resulting in a decrease in endogenous insulin secretion and paves ways for the decreased utilization of glucose by body tissues [20]. It results in elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides [21].

Materials and Methods

Plant Material

The onion bulbs of *A. cepa* were purchased in Kaduna local market, Kaduna North Local Government area, Kaduna state, Nigeria in December, 2015. The onion bulbs were washed with clean water to remove dirt, boiled until done, air dried for two weeks and pulverized to coarse powder using pestle and mortar in the laboratory. The powdered material was weighed and kept in an airtight polyethene bag.

Sample Extraction

500 g of the powdered plant material was macerated sequentially with ethanol (1.5 L) at room temperature (27^{o}C) in a maceration bottle. The maceration bottle was sealed properly to prevent evaporation and was shaken from time to time for four days to obtain a substantial amount of extract and then decanted and filtered using filter paper. Fresh solvent (1.5 L) was added to there mains and the bottle occasionally shaken for two days. The extract was decanted and again filtered into the previous filtered extract. The filtrate was then concentrated using rotary evaporator to obtain a brownish-black solid ethanol extract and the solvent recovered.

Phytochemical Screening

Phytochemical screening was carried out on the ethanol extract of *Allium cepa* L. using standard procedures and tests [22-23] to determine the presence of alkaloids, flavonoids, steroids, terpenoids, tannins, cardiac glycosides, reducing sugar and saponins.

Pharmacological Study

Animals

All experimental animals used were male and female albino mice (weighing between 22-32g and were obtained from Nigerian Institute for Tripanosomiasis Research (NITR), Kaduna State, Nigeria. The mice were kept in clean and dry aluminium cages under standard environmental conditions and were fed using growers marsh feed and given water *adlibitum*. The mice were fasted, however, for 12 hours before experimentation. The experimental methods involving animals have been approved by Animal Research Ethics Committee of Kaduna State University, Kaduna-Nigeria.

Experimental design

Diabetes was induced by a single intraperitoneal injection of 150mg/kg body weight alloxan monohydrate freshly dissolved in regular saline 0.9% physiological saline immediately before used to overnight fasted albino mice (free access to water was allowed). Seven days after, animals with fasting blood glucose level ≥7.0 mmol/dL(or ≥126 mg/dL) or more were considered diabetic and employed in the study.The diabetic mice were then grouped into 5 groups of five mice each as follows:

Group I: Served as positive control and received glibenclamide (2 ml/kg body weight)
Group II: Received ethanol extract at 150 mg/kg body weight
Group III: Received ethanol extract at 450 mg/kg
Group IV: Received ethanol extract at 750 mg/kg
Group V: Served as negative control receiving physiological saline (10 ml/kgbody weight)

The animals were treated once and blood glucose concentrations were measured at 0, 12, 24, 48 and 72 hours. Blood samples were taken by a snip-cut at the tip of the tail and blood glucose levels were measured with a glucometer (a ONE TOUCH Ultra easy blood glucose monitoring system, LifeScan Europe Division of Cilag GmbH international 6300 Zug Switzerland).

Statistical Analysis and Data Evaluation

All the values of blood glucose concentrations were expressed as mean ± standard error of mean (SEM.) and analyzed for ANOVA followed by Student's t-test. Differences between groups were considered significant at 5% level of significance i.e. $P \leq 0.05$.

Results and Discussion

Phytochemical screening

The results of phytochemical studies on the ethanol extract of cooked *Allium cepa* have been provided in table 1.0.

Table 1.0: Phytochemical analysis of the extract of cooked*Allium cepa*

Phytochemicals	Observation
Alkaloids	++
Flavonoids	++
Steroids	+
Terpenoids	+
Tannins	-
Cardiac Glycoside	+*
Reducing sugar	-
Anthraquinones	+
Saponins	-

Key: + = positive,- = negative, +* = slight positive, ++ = strongly positive

The results of phytochemical screening of the ethanol extract of cooked onion bulbs have indicated the presence of alkaloids, flavonoids, steroids, anthraquinones, terpenoids and cardiac glycoside. These phytochemicals except anthraquinones were indicated present in both the raw and boiled onion bulbs [24]. So many other phytochemicals

have been isolated from onion and these include various monosaccharides, organosulfur compounds, S-alk(en)yl cysteine sulfoxides, and seleno compounds [25-27].

Pharmacological study

The effects of the ethanol extracts of the bulb of *Allium cepa* on fasting blood glucose levels in alloxan monohydrate-induced diabetic albino mice have been given in table 2.0.

Table 2.0: Antidiabetic effect of ethanol extracts of cooked *Allium cepa*in alloxan-induced diabetic mice

Test material	Groups	Blood glucose levels in (mmol/L) sampling time in hours				
		0	12	24	48	72
Glibenclamide (positive control)	I	9.5±2.2	6.6±1.8	6.0±3.0	5.8±2.2	4.4±0.3
150 mg/kg	II	9.4±5.8	8.2±4.4	7.4±4.1	6.7±3.3	5.9±2.4
450 mg/kg	III	13.5±1.2	10.4±0.8	9.0±0.3	7.6±0.7	6.2±2.3
750 mg/kg	IV	10.8±2.3	8.7±2.1	6.9±2.7	6.0±2.2	5.4±2.5
Physiological saline (negative control)	V	13.8±3.2	12.2±1.4	13.1±1.3	12.9±1.8	12.5±2.4

Values of blood glucose levels are given as mean ± SEM (n = 5), P<0.05 with respect to both Glibenclamide and physiological saline. The data was analyzedfor ANOVA followed by student's t-test

Comparison of the average values of blood glucose levels in the treated and untreated (control) groups of alloxan-induced diabetic mice suggested some favourable antidiabetic effect of *Allium cepa*. Statistical analysis using student's t-test revealed that there is a statistically valid difference between the treated and the control groups. The ethanol extracts exhibited significant (P<0.05) anti-diabetic effect at 48 h and 72 h at the doses of 450 and 750 mg/kg, respectively. From the results it can be suggested that the ethanol extract exhibited dose dependent action in a similar mechanism as glibenclamide i.e., by stimulation of surviving β-cells to release more insulin [28].

The antidiabetic activities of the graded extracts tested in this study could be attributed to the phytochemicals found in this extract, some of which have been reported to be hyperglycaemic by many researchers. Terpenoids have been shown to lower blood glucose in rats [29]. Two anthraquinones isolated from the ethanol extract of rhubarb rhizome have been reported to have anti-diabetic properties [30]. Alkaloids have also been implicated to have hypoglycaemic activity and it was found that the neem seed kernel powder contained hypoglycaemic alkaloids that lowered blood sugar levels in alloxan-induced diabetic rabbits [31].The various sulphur-containing compounds produced by onions, are claimed to be responsible for the distinctive odour, flavour, and lachrymatory properties of onions [32].S-methylcysteinesulphoxide isolated from onion has also been reported to have anti-hyperglycemic effect [33] although as compared to the standard drugs, glibenclamide and insulin theantidiabetic activity was found to be less [17].In another report onions have been found to be hypoglyceimic and allyl propyl disulphide is implicated to be the active principle it said to lower blood sugar levels by increasing the amounts of free insulin available [34]. Flavonoid-rich fractions have been isolated from two plants, *Psidium guajava* leaves and *Tamarindus indica.* The *Psidium guajava* leaves fraction was found to have lowered blood glucose in humans [35]. The *Tamarindus indica* fraction, when tested in both the alloxan- and fructose-induced hyperglycaemia in rats, showed a significant ($p < 0.05$) reduction in elevated blood glucose level at dose- and time-dependent manner [36].

Allium cepa (onion) is a major source of flavonoids and the two major ones are the quercetin glycosides, quercetin 4'-O-beta-glucoside and quercetin 3,4'-O-beta-diglucosides (a flavonol glycoside) [37].These quercetin derivatives are been regarded as the most important flavonoids to improve diabetic status in animal models [24]. Onion is cooked in various ways such with water alone, frying with oil or microwave cooking. Various cooking methods do not consider the degradation of quercetin glycosides when cooking onion. Microwave cooking without water better retains flavonoids and also frying with oil does not affect flavonoid intake. However, boiling of onion leads to about 30% loss of quercetin glycosides, which are transferred to the boiling water [7]. Quercetin glycosides are therefore heat-stable and transferable to cooking water. The hydrolysis of quercetin glycosides for daily cooking might occur with the addition of seasonings such as glutamic acid [38]. More recently, tremendous studies have found that

flavonoids originated from foods could improve glucose metabolism and protect human beings from diseases like obesity, diabetes and their complications [39].

Conclusion

In conclusion, the present study suggests that even after cooking the onion bulb contains ingredients capable of treating diabetes. From the experimental results, it can be suggested that, the ethanol extract of the cooked onion bulbs exhibited dose dependent action in a similar mechanism as glibenclamide *i.e.*, by stimulation of surviving beta cells to release more insulin. However, comparative studies in this potentiality between the cooked onion bulb and the resulting water used in cooking the bulbs will be of interest.

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