

Full Length Research paper

Phytochemical screening and hypoglycemic effect of methanolic fruit pulp extract of *Cucumis sativus* in alloxan induced diabetic rats

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Phytochemical screening and hypoglycemic effect of methanolic fruit pulp extract of *Cucumis sativus* on alloxan induced diabetic rats was investigated. The phytochemical analysis of the methanolic fruit pulp extract of *C. sativus* indicated the presence of saponins, glycosides, terpenes, phenolics, alkaloids, flavonoids, and tannins. The methanolic fruit pulp extract of *C. sativus* was administered orally to the alloxan induced diabetic rats and it significantly decreased ($P < 0.05$) the fasting blood glucose concentration (mg/dl) from 231.25 ± 1.11 to 82.25 ± 1.55 at 500 mg/kg body weight. The standard antidiabetic drug (Glibenclamide) administered orally at 5 mg/kg body weight also significantly decreased ($P < 0.05$) blood glucose concentration from 189.00 ± 2.42 to 61.00 ± 2.48 . This study therefore revealed that the methanolic fruit pulp extract of *C. sativus* contains active substances with hypoglycemic activity and could be used in the treatment and management of diabetes mellitus.

Key words: *Cucumis sativus*, hypoglycemic activity, phytochemical screening, alloxan, methanolic.

INTRODUCTION

Diabetes mellitus is a heterogeneous group of chronic disorders of carbohydrate, lipid and protein metabolism characterized by high blood glucose levels due to relative or absolute deficiency of insulin (Eiselein et al., 2004).

Diabetes mellitus affects more than 100 million people worldwide and the number of people with diabetes is increasing due to population growth, aging and increasing prevalence of obesity and physical inactivity (Nair et al., 2006). In 2010, World Health Organization (WHO) estimated that 285 million people were living with diabetes (corresponding to 6.4% of the world's adult

population). About 7 million people develop the disease each year and 3.9 million deaths were attributed to diabetes yearly (Shaw et al., 2010). Current predictions estimate that the prevalence of diabetes will reach 438 million by 2030 (corresponding to 7.8% of the adult population) and that 80% of prevalent cases will occur in the developing world (Roglic and Unwin, 2010).

Phytochemicals are substances found in edible fruits and vegetables that exhibit a potential for modulating human metabolism in a manner beneficial for the prevention of chronic and degenerative diseases (Tripoli

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et al., 2007). Plants usually produce such low-molecular-weight ingredients for their protection against pests and diseases, for the regulation of their growth, or as pigments, essence, or odor. Scientists have identified thousands of phytochemicals, including flavonoids, anthraquinone, glucosinolates (isothiocyanates and indoles), phenolic acids, phytates, and alkaloids in vegetables, fruits, grains, legumes, and other plant sources (Perez et al., 2006).

Some phytochemicals in diverse plants have beneficial health effects such as anti-obesity, lipid-lowering, and antidiabetic properties. The vast study done on cucumber proved that the plant has many important phytoconstituents like glycosides, flavonoids, terpenes, sterol, saponins, and tannins (Kren and Ludmila, 2001). These compounds were found to be responsible for the pharmacological activity (Jony et al., 2013). Terpenoids are known to possess medicinal potency against inflammation, cancer, malaria, cholesterol synthesis inhibition, viral and bacterial agents (Mahato and Sen, 1997). Also, the alkaloids are used as anaesthetic agent. Although, variations have been noticed in the leaf alkaloid content of androgenic diploid plants of *Datura innoxia* (Herouart et al., 1988, Kukreja and Maclaren, 1999). Phenolic compounds are known to decrease cholesterol and triglyceride levels in rats (Gutierrez-Lugo et al., 1996, Argueta, 1994). The tannins are known to inhibit microbial activities, the mechanism is based on its ability to bind proteins thereby inhibiting cell protein synthesis (Ayepola and Adeniji, 2008). The treatment of diabetes with synthetic drugs many years back has not led to any proper drug for the treatment of diabetes, because they are not affordable and have side effects (Sharmin et al., 2013). For this reason, traditional medicinal practitioners have described a number of plants used as complementary herbal antidiabetic drugs, mostly with less dangerous side effects and low cost (Singh et al., 2009). The hypoglycemic effect of several of such plants belonging to families Leguminosae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Araliaceae (Bnouham et al., 2006) has been confirmed, and the mechanisms of hypoglycemic activity of these new bioactive drug constituents are also being studied (Jung et al., 2006). Plant possesses various phytoconstituents which seems to be the active hypoglycemic principles, suggests different sites of action within the body. Most of the researches are carried out to evaluate the therapeutic effect of the plants along with their mode of action (Colaguirri, 2010). Plants show antidiabetic activity with various mechanism, like alteration of glucose metabolism, insulin-like effect, improve glucose tolerance, reduction of absorption of glucose from intestine, enhancing insulin signal pathway, hypoglycemia through increase glucose uptake and glycogen synthesis, generation of beta cell in pancreas (Banshidhar and Deepmala, 2013).

Cucumber is a widely cultivated plant in the gourd

family Cucurbitaceae. It is a creeping vine that bears cylindrical fruits that are used as culinary vegetables.

Within the varieties of cucumber, several different cultivars emerged. The plant has large leaves that form a canopy over the fruit. They have an enclosed seed, hence they are classified as accessory fruits. Much like tomatoes and squash, they are prepared and eaten as vegetables (Tindall, 1975).

Several plants of the Cucurbitaceae family are established for their hypoglycemic properties. Cucumber (*Cucumis sativus*) originated in India, but now cultivated in different parts of the world. In Mexico, cucumber is one of the edible plants with hypoglycemic potentials. The plant part in use currently against diabetes includes the seeds, pulp and the fruit itself. Antihypoglycemic study of this plant was studied in healthy rabbits which significantly lowered the blood glucose level (Stano et al., 2002). In addition, the plant has medicinal potency against hypercholesterolemia.

C. sativus is an important medicinal plant with diverse pharmacological activities such as antibacterial, antifungal, antidiabetic, cytotoxic, antacid and carminative activity, hepatoprotective activity, wound healing activities; hence, this plant provides a significant role in the prevention and treatment of a disease (Jony et al., 2013). This study will therefore determine the phytoconstituents and hypoglycemic effect of the methanolic extract of *C. sativus* fruit pulp in alloxan induced albino rats.

MATERIALS AND METHODS

Apparatus

The apparatus used were glucometer (Accu-check Active-Mannheim Germany), glucose strips (Accu-check strips), Reflux extractor, water bath and blender.

Sample collection and preparation

C. sativus was purchased in January from old market in Lokoja, Kogi State. The collected sample was washed, cut into small pieces, dried completely under the controlled mild sun temperature of 25°C and ground with an electric grinder. 50 grams of the powdered fruit pulp of *C. sativus* was weighed and 400 ml of methanol was measured in a round bottom flask and placed into a reflux extractor at 65°C for 3 h after which it was filtered through filter paper (Whatman filter paper no. 1). The methanolic solution was allowed to evaporate using water bath until dried extract was obtained (Sokeng, 2007).

Chemicals and reagents

The chemical used were methanol (AR), alloxan, chloroform, Wagners reagent and acetic anhydride.

Experimental animals

Albino rats of either sexes weighing between 130 and 220 g were

housed in plastic cage and the rats were acclimatized in the laboratory for a period of two weeks with adequate food and water.

Experimental design

Sixteen rats were randomly grouped into four groups with four rats in each group and designated as:

Group 1: Non induced; Group 2: Alloxan-induced diabetic rats not treated; Group 3: Alloxan- induced diabetic rats treated with 500 mg/kg body weight of extract; Group 4: Alloxan-induced diabetic rats treated with glibenclimide. All the treatments were administered in a suitable vehicle of 1% dimethyl sulphoxide (DMSO).

Experimental rats' induction

The fasting blood glucose concentration (mg/dl) of the rats was tested before induction, then a prepared solution of alloxan monohydrate (100 mg/kg body weight) was administered intraperitoneally into groups 2, 3 and 4. After two days interval of induction, the rats were tested and rats with blood glucose levels above 150 mg/dl (60% of the rats) were used to confirm diabetes. Treatment with extract and glibenclamide followed for a period of twenty one days (Adeneye and Agbaje, 2008).

Phytochemical screening

The *C. sativus* fruit pulp extract was screened for phytochemical properties by Trease (1983) and Sofowara (1983).

Anthraquinone test

Five milliliters of the extract was added to 10 ml of benzene. Five milliliters of 10% $\text{NH}_3(\text{aq})$ was added and mixed. The presence of anthraquinone is indicated by a pink/violet color in the ammonia phase at the bottom of the test tube (Sofowara, 1983).

Alkaloid test

Two milliliters of the extract was treated with 10 ml of 1% HCL in water bath for 30 min. The solution was then treated with few drops of Wagners reagent and the colour change was observed. The presence of alkaloid is indicated with precipitate formation (Sofowara, 1983).

Cardiac glycoside test

Two milliliters of chloroform was used to dilute 0.5 ml of extract. Sulphuric acid was carefully added to the solution drop wise. At the chloroform/sulphuric acid interphase, a reddish coloration indicates the presence of cardiac glycosides (Trease, 1983).

Saponin test

0.5 ml of extract was mixed with 10 ml of distilled water. Frothing which persist on warming of the test tube confirms the preliminary evidence for the presence of saponin (Sofowara, 1983).

Steroid test

Five drops of concentrated sulphuric acid were added to 2 ml of the extract. A reddish brown coloration indicates the presence of

steroids (Trease, 1983).

Phenol test

Two milliliters of extract was added to 2 ml of ferric chloride solution. A deep bluish green solution was formed with the presence of phenol (Sofowara, 1983).

Tannin test

Three milliliters of the extract was added to 5 ml of distilled water and then heated in water bath. To this solution, FeCl_3 solution was added. A blue-black or green precipitate indicated the presence of tannins (Trease, 1983).

Flavonoid test

One milliliter of the extract was added to 5 ml of distilled water and then filtered to obtain 2 ml of the filtrate. A few drops of 10% ferric chloride solution were added, blue-violet coloration was an indication of the presence of flavonoid (Trease, 1983).

Terpene test

One milliliter of the extract was added to 5 ml of chloroform in a test tube. 3 ml of acetic anhydride was added to the chloroform-extract to which 2 ml of concentrated sulphuric acid was also added. The formation of a ring at the interphase between the two immiscible liquids is a preliminary evidence of the presence of terpenes (Trease, 1983).

Oral administration of plant extract

500 mg/kg body weight of the extract was administered to group 3 alloxan induced diabetic rats and 5 mg/kg body weight of standard drug (glibenclamide) to group 4 alloxan-induced diabetic rats once each day.

Determination of fasting blood glucose level

The fasting blood glucose was determined using glucometer kit (accucheck) after overnight fasting for about 8 to 10 h. The tail was punctured and the blood from the tail was dropped on the strip which had been inserted into the glucometer to obtain the blood glucose concentration in mg/dl for each rat in all the groups at an interval of days 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21.

Statistical analysis of results

Statistical comparisons were performed by one-way analysis of variance (ANOVA), and Dunnett's t-test for comparison. Results were considered significant when p values were less than 0.05 ($p < 0.05$).

RESULTS

The results are as shown in Table 1 and Figure 1, respectively. Table 1 shows the phytoconstituents of methanolic extract of *C. sativus* fruit pulp and Figure 1

Table 1. Phytoconstituents of methanolic extract of *Cucumis sativus* fruit pulp.

Compound	Inference
Anthraquinones	-
Alkaloids	+
Cardiac glycosides	+++
Saponins	+
Steroids	-
Phenols	++
Tannins	+
Flavonoids	+
Terpenes	+++

+: Slightly present, ++: Moderately present, +++: Highly present, -: Not detected.

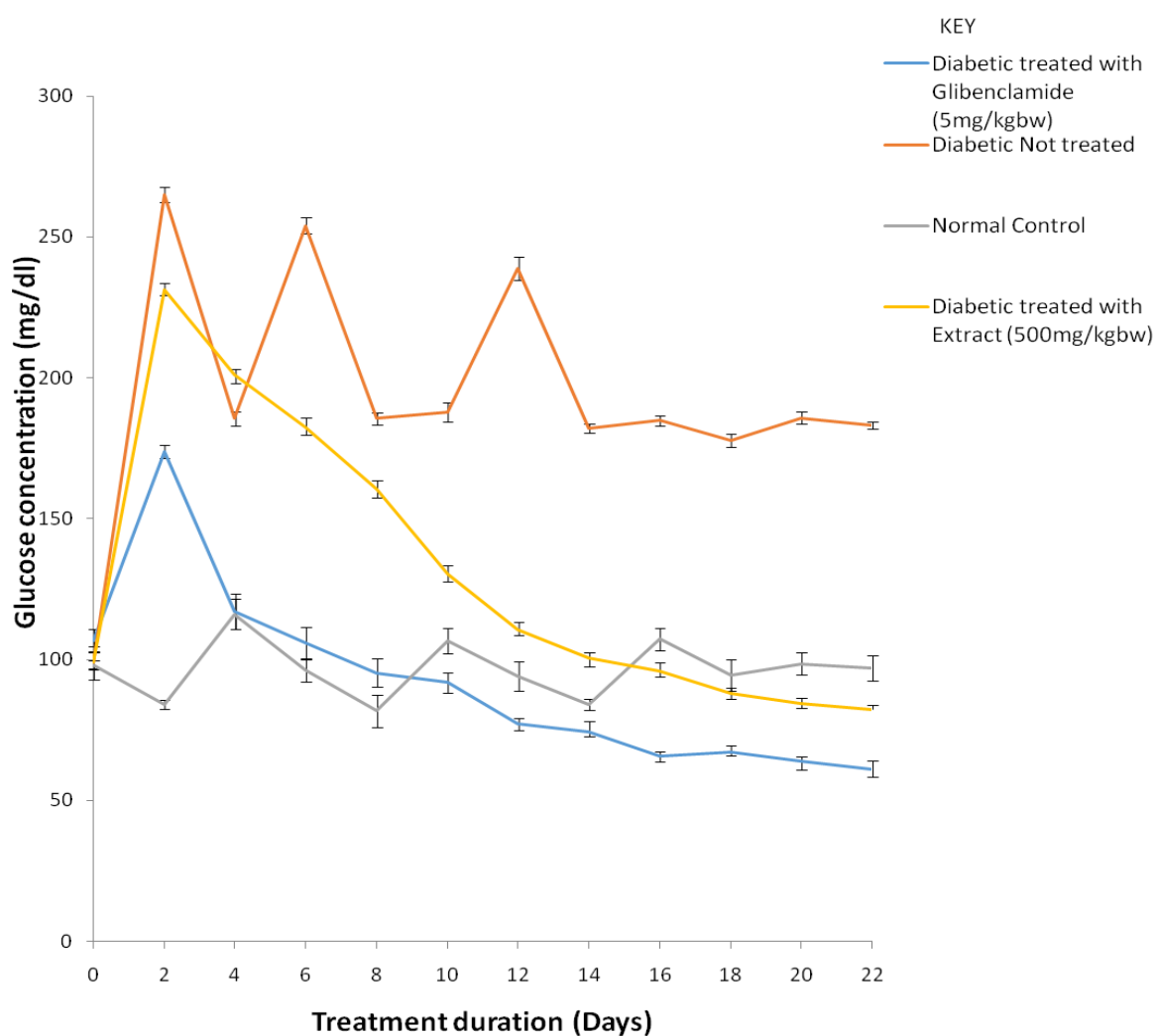


Figure 1. Fasting blood glucose concentration (mg/dl) of diabetic rats not treated and non induced, treated with extract and standard drug.

shows the fasting blood glucose levels of diabetic rats not treated and non induced, treated with extract and standard drug.

DISCUSSION

Table 1 reveals that the phytochemical analysis of

methanolic fruit pulp extract of *C. sativus* contains important phytoconstituents like tannins, saponins, terpenes, glycosides, alkaloids, flavonoids and phenols, while anthraquinone and steroids are absent. The seed extracts of *C. sativus* have also been reported to contain these phytochemicals (Ankita et al., 2012). Flavonoid and tannins have been reported to cause regeneration of damaged pancreatic islets, stimulate calcium and glucose uptake (Tapas et al., 2008, Kumar and Clark, 2002). These compounds are known to be responsible for the hypoglycemic activity of the plant as compared with other hypoglycemic plants which contains similar phytoconstituent found in *Luffa acutangula* fruit extract (Pimple et al., 2011) and methanolic root bark extract of *Acacia albida* (Salisu et al., 2009). It has been reported that *C. sativus* seeds are found as suitable food for medicinal purposes against some diseases such as diabetes, hyperlipidemia, hypertension, gall bladder stones, constipation, dyspepsia in Asian traditional remedies (Trease and Evans, 2002; Roman-Romos et al., 1995; Amin, 2005).

The results in Figure 1 reveals that oral administration of 500 mg/kg body weight of *C. sativus* methanolic fruit pulp extract caused a significant decrease ($p < 0.05$) in fasting blood glucose concentration of alloxan-induced diabetic rats from 231.25 ± 1.11 to 82.25 ± 1.55 and oral administration of 5 mg/kg body weight of standard drug (glibenclamide) caused a significant decrease ($p < 0.05$) in fasting blood glucose concentration of alloxan-induced diabetic rats from 189.00 ± 2.42 to 61.00 ± 2.48 , respectively, while the induced not treated remained hyperglycemic from 265.00 ± 2.86 to 183.00 ± 1.30 and the non induced from 98.00 ± 1.47 to 97.00 ± 4.52 after twenty one days. It has been reported that fractions of *C. sativus* seed extract were effective to cause hypoglycemia in normal group even after prolonged treatment during sub-acute phase of the study.

This corroborated the findings by Chandrasekar et al. (1989) who investigated blood glucose lowering effect of eight plants of Cucurbitaceae family including *C. sativus* fruit extract. This study suggests that the methanolic extract of *C. sativus* fruit pulp has hypoglycemic effect by reducing fasting blood glucose concentration and could be as effective as glibenclamide used as hypoglycemic standard drug.

This study reveals similar hypoglycemic effects with the ethanol extracts of Cucurbitaceae family fruits (Sharmin et al., 2013) and *Viscum album* extract (Shahabuddin et al., 2011) on alloxan-induced diabetic rats.

Conclusion

From this study, it can be concluded that the methanolic fruit pulp extract of *C. sativus* at 500 mg/kg body weight is an active hypoglycemic remedy and may be used in the treatment and management of diabetes mellitus since it has been demonstrated in rats. However, further

research should be carried out to uncover the precise mechanism of action.

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Conflict of Interest

The authors declare no conflict of interest.

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