

In Vitro Carbohydrate Digestibility of Copper Nanoparticulated Bitter Gourd Extract

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Abstract

Diabetes mellitus is a multifunctional disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion resulting in impaired insulin action. The intestinal digestive enzymes α -glucosidase and α -amylase play a key role in carbohydrate digestion; one main anti-diabetic approach is to reduce the post prandial glucose level in blood by inhibition of α - glucosidase and α - amylase enzymes. Copper nanoparticles prepared by green synthesis from ethanol extract of bitter gourd were investigated for its carbohydrate digestibility efficacy. The assay results of copper nanoparticles showed dose dependent activity against α - amylase enzyme with an IC₅₀ value of 59.89 μ g/ml \pm 0.01 μ g/ml. Similarly dose dependent activity was exhibited by α - glucosidase enzyme by the green synthesized nanoparticles with an IC₅₀ value of 55.79 μ g/ml \pm 0.16 μ g/ml. Therefore, it was suggested that colloidal CuNps could be used as an effective material for treatment of diabetes by controlling blood glucose level.

Keywords: Diabetes; Nanoparticulated; Carbohydrate; Hyperglycemia

Introduction

Diabetes mellitus is a major global health concern with a projected rise in prevalence from 171 million in 2000 to 366 million in 2030 [1]. It is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels called hyperglycemia [2]. Being a major degenerative disease, diabetes is found in all parts of the world and it is becoming the third most lethal disease of mankind and increasing rapidly [3]. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million individuals worldwide. Diabetes has been a clinical model for general medicine [4]. Complementary and alternative medicine involves the use of herbs and other dietary supplements as alternatives to mainstream western medical treatment. A recent study has estimated that up to 30% of patients with diabetes mellitus use complementary and alternative medicine [5].

Momordica charantia (*M. charantia*), also known as bitter melon, karela, balsam pear or bitter gourd, is a popular plant used for the treating of diabetes-related conditions amongst the indigenous populations of Asia, South America, India, the Caribbean and East Africa [6]. Its fruit has a distinguishing bitter taste, which is more pronounced as it ripens, hence the name bitter melon or bitter gourd. Biochemical and animal model experiments have produced abundant data and hypotheses accounting for the anti-diabetic effects of *M. charantia*.

Nanotechnology is promising as a rapidly growing field with its application in science and technology. Noble metal nanoparticles such

as silver, gold and platinum are broadly applied in medicinal applications. Copper nanoparticles are important materials that have been studied widely. There is a growing need to develop an eco-friendly method for the synthesis of nanoparticles that does not utilize toxic chemicals. In general, nanoparticles are prepared by a variety of physical and chemical methods which are not eco-friendly [7]. Nowadays, green chemistry procedures are using various biological systems such as bacteria, fungi, yeast and plant extract for the synthesis of nanoparticles [8].

In the present work, use of clear ethanol extracts of fruit of *Momordica charantia* which contains an array of biologically active plant chemicals including triterpenes, proteins and steroids) was tried and the *in vitro* anti-diabetic activity subsequently investigated which is reported in this study.

Materials and Methods

Sample preparation

One g of dried powder in 50.0 ml of ethanol was subjected to exhaustive extraction by cold maceration for 72 h. The conical flasks were sealed to avoid evaporation. The slurries were centrifuged at 3,000 rpm for 10 min and filtered through Whatman No. 40 filter paper to obtain a clear extract. 90 ml of 0.001 M copper nitrate mixture was mixed with a 10 ml of ethanol extract and the mixture was incubated at room temperature for 24 h. The slight color change of copper nitrate indicates the formation of copper nanoparticles through reduction of copper ions from Cu⁺² to Cu. The samples were then centrifuged at 4000 rpm for 15 min to get a clear supernatant.

Characterization of nanoparticles

They were subsequently characterized using UV - Visible Spectrophotometer to record the localized surface plasmon resonance of copper nanoparticles at 200 cm^{-1} - 800 cm^{-1} . The size and morphology was examined using Scanning electronic Microscopy (SEM), Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) techniques. FTIR spectrum was recorded in mid IR region in the range of 400 - 4000 wave number (cm^{-1}). The structure of the nanoparticles was obtained using X-ray diffraction (XRD) technique (Figure 1).

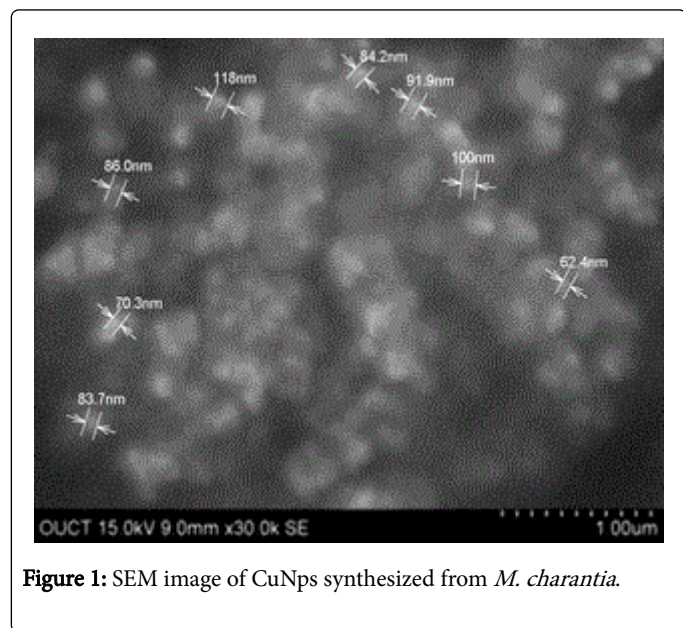


Figure 1: SEM image of CuNps synthesized from *M. charantia*.

Inhibition of α -amylase enzyme activity [9]

Two experimental models were used to evaluate the anti-diabetic activity, i.e., inhibition of α - amylase and α - glucosidase activity as these enzymes influence the rate of carbohydrate digestibility in the body.

Various concentrations (10, 20, 40, 60, 80, 100 $\mu\text{g/ml}$) of the samples were dispersed in 1.0 ml of 2 M phosphate buffer (pH 6.9) with addition of enzyme buffer of 0.5 ml was added to the sample suspension and incubated at 37°C for 2 h. After the incubation period, 2 ml of 3, 5 - dinitrosalicylic acid reagent was quickly added to all the mixture dispersions and heated for 5 min. After cooling, the solution was made up to 25.0 ml with distilled water and filtered. The absorbance was measured at 550 nm. A sample blank and 4.0 ml of maltose standard were run simultaneously with the samples. The values were expressed as mg of maltose released / g sample.

Inhibition of α - glucosidase enzyme activity

The inhibitory activity of α -glucosidase enzyme was determined by incubating 1.0 ml of starch substrate (2% w/v maltose or sucrose) with 0.2 ml Tris buffer (pH 8.0) and various concentrations of samples for 5 min at 37°C. The reaction was initiated by adding 1.0 ml of α - glucosidase enzyme (IU/ml) to it, followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2.0 ml of 6 N Hydrochloric acid. The intensity of colour was measured at 540 nm and repeated thrice consecutively.

The anti-diabetic activity was also expressed as IC₅₀ from the graph plotted with the average of the three observations.

Result and Discussion

In this study, the ability of the extracts to inhibit the crude extracts and synthesized copper nanoparticles were compared on the basis of percentage inhibition and IC₅₀ values as shown in Figures 2 and 3 and Table 1. The various samples showed a dose dependent inhibition of α -amylase enzyme activity. However, maximum inhibition (76.93%) was recorded for CuNps synthesized from ethanol extract while the least inhibitory activity was observed for crude extract (60.38%). The standard drug acarbose which was used as a reference agent had a value of 75.10%. The IC₅₀ for crude extracts, CuNps and acarbose was 81.34, 57.49 and 59.89 $\mu\text{g/ml}$ respectively. Ethanol extracts CuNps showed significantly higher ($P < 0.05$) inhibitory activity against α -amylase enzyme when compared to the standard drug acarbose due to the presence of substantial amount of charantin, the main bioactive component present in bitter gourd and responsible for its ability to ameliorate diabetic conditions as insulin in the body.

Similarly, crude extracts of bitter gourd and the mediated copper nanoparticles showed a dose dependent inhibitory activity against α -glucosidase enzyme activity. The maximum percentage inhibitory activity was recorded for CuNps ethanol extract and acarbose with inhibition of 80.3% and 77.17% respectively. The least inhibitory activity was recorded for the crude extracts (62.96%) at a maximum concentration of 100 $\mu\text{g/ml}$. The percentage inhibition against α - glucosidase enzyme activity was also plotted against concentration to deduce IC₅₀ by linear regression analysis with values of 72.81 $\mu\text{g/ml}$, 57.25 $\mu\text{g/ml}$ and 55.79 $\mu\text{g/ml}$ for crude extracts, acarbose and CuNps respectively.

Synthetic α - amylase inhibitor such as acarbose is complex oligosaccharides that delay the digestion of carbohydrates and has been in use over the years. It inhibits the breakdown of starch by pancreatic amylase. But it's been reported that these synthetic inhibitors cause side effects such as abdominal pain, diarrhea and soft faeces in the colon. Many natural resources have been reported for their anti-diabetic activities in Ayurveda but have not gained much importance as synthetic medicines due to lack of sustained scientific evidence about possible mechanisms through which these herbs can act to control the blood glucose level [10]. One of such mechanisms is an alteration of the activity of glucose metabolism by α - amylase and α - glucosidase inhibitors (plant extracts) acting as anti-nutrient that obstructs the digestion and absorption of carbohydrates [11].

S. No.	Sample	IC ₅₀ ($\mu\text{g/ml}$) values	
		Inhibition of α -amylase activity	Inhibition of α -glucosidase activity
1	Crude ethanol extract	81.34 \pm 0.04 ^a	72.81 \pm 0.12 ^a
2	Standard acarbose	57.49 \pm 0.01 ^c	57.25 \pm 0.08 ^b
3	CuNps ethanolic extract	59.89 \pm 0.01 ^b	55.79 \pm 0.16 ^c

Table 1: IC₅₀ values of extracts and nanoparticles against selected enzymes.

The mechanism of this exerted action may be due to its activity on Carbohydrate binding regions of α - glucosidase enzyme, α - amylase and endoglucanases that catalyze the hydrolysis of internal α - 1, 4 glucosidic linkages in starch and other related polysaccharides targeted for the suppression of postprandial hyperglycemia. These enzymes are responsible in hydrolyzing dietary starch into maltose which was then broken down to glucose prior to absorption [12,13].

In this study, bitter gourd mediated copper nanoparticles were a more potent in inhibiting α -amylase activity than acarbose. Hence, it may be concluded that bitter gourd due to its inhibitory activity on carbohydrate binding regions of α -glucosidase enzyme, α -amylase and endoglucanases that catalyze the hydrolysis of internal α - 1, 4 glucosidic linkages in starch and other related polysaccharides targeted for the suppression of postprandial hyperglycemia α -amylase enzyme correlates with earlier studies done by researchers which reported that the plant material has potent activity against diabetes.

Inhibitors of intestinal α -glucosidase enzymes retard the rate of carbohydrate digestion, thereby providing an alternative therapeutic option for modulation of postprandial hyperglycemia (PPHG) [14]. In diabetic patients, a sustained reduction of hyperglycemia was to decrease the risk of developing micro-vascular and macro-vascular diseases and their associated complication [2]. CuNps synthesized from bitter gourd had higher biological properties and improved activity of due to their high surface area to volume ratio, thus increasing the surface area (promoting the electron transfer reaction) and might increase the pharmacokinetics from a biological view point.

Therefore, it was suggested that colloidal CuNps could be used as an effective material for treatment of diabetes after in vivo pharmacokinetic studies. The high in-vitro hypoglycemic assessment of bitter gourd in ethanol extracts and its nanoparticles could be possibly due to substantial liberation of phytochemical compounds such as phenolics, vicine, alkaloids, charantin, polypeptide-p, cryptoxanthin, cucurbitins, diosgenin, elaeostearic acids, erythrodiol and galacturonic acids [15].

Note: Values are expressed as mean \pm standard deviation of three determinations. Mean values with similar superscripts within a column row do not differ significantly ($P < 0.05$) [16].

Conclusion

To date, *M. charantia* has been extensively studied worldwide for its medicinal properties to treat a number of diseases. It is described as a versatile plant worthy of treating almost any disease inflicted on mankind. This may be due to the fact that the plant possesses over 225 different medicinal constituents. Bioassay method was done to check the anti-diabetic property of bitter gourd mediated copper nanoparticles. It showed the better significant effect on inhibition of α - amylase and α - glucosidase.

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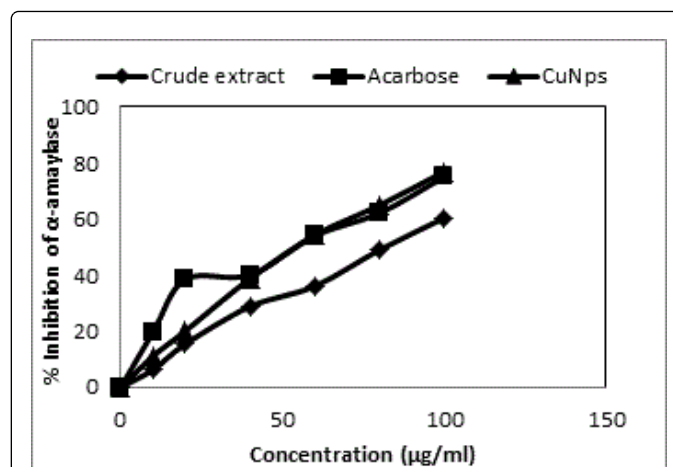


Figure 2: Percentage inhibition of α - amylase enzyme activity.

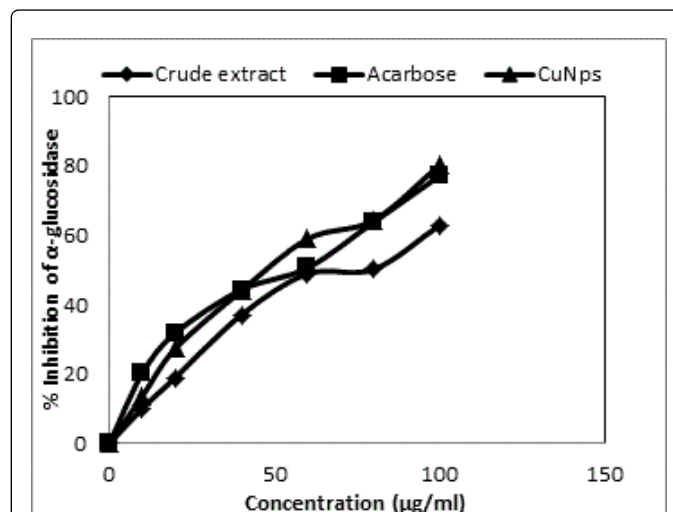


Figure 3: Percentage inhibition of α - glucosidase enzyme activity.

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