Research Article

Evaluation of Therapeutic Effect of Mg doped ZnS Nano Particle and *Bougainvillea glabra* Flower Fraction on HepG2 Cell Lines

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ABSTRACT

Diabetes mellitus is chronic disease which causes ill effects on human health. Over many years, a number of research works undergone to control diabetes mellitus among humans. Most of the researchers contributed various drugs for controlling diabetes mellitus. It is observed that drugs either causing side effects or efficiency is very less. Hence, the present study is formulated to find out efficient drug to control diabetes mellitus, which is free from side effects. Zinc has potential antioxidant activity. Hence it is selected as drug delivery agent as in the form of Mg doped ZnS nanoparticle. Herbal formulations always showed potential biological activities. and herbal delivery with nanoparticle showed enhanced antidiabetic activity. The Mg doped ZnS nanoparticle and *Bougainvillea glabra* flower fraction were evaluated for their cytotoxic nature against HepG2 cell lines. Glucose uptake activity and α-Glucosidase activity were also estimated. The results showed potential antidiabetic activity and antioxidant activity with negligible cytotoxicity, signifying that Mg doped ZnS nano particle and *Bougainvillea glabra* flower fraction as potential candidate for the treatment of diabetes.

Key words: *Bougainvillea glabra*, Mg doped nanoparticle, Cytotoxicity, Antioxidant activity, HepG2 cell lines.

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INTRODUCTION

Diabetes almost affects 6% population in worldwide.[1] The patients need continuous medication to maintain the blood glucose level. Prolonged medication will make a bad impact on economy of the people. Developing countries like India very much suffers due to increasing incidence of diabetes.[2] The world population suffers from diabetes mellitus due to their sedentary lifestyle and food habits. It is a growing need to identify the toxic free fast acting drug to control diabetes mellitus. Increased blood glucose level is one of the major cause of diabetes mellitus which already known as metabolic disorder. Throughout the world many number of people suffered by diabetes by their imbalanced diet.[3] Diabetes mellitus causes serious health issues from well developed countries into under developed countries. Herbal plants have acts as promising antidiabetic agent and easy to utilizing *invivo* practices that could helps to overcome adverse effects of allopathic drugs. The plant based treatments are considered as ancient style of medicine which gets best results in now a days.[4] The nanoparticles are attains greatest interest over scientist due to their physical and chemical properties with size dependent nature.[5] Generally all living cells are very tiny in size for their functional purpose like cells 10 μm diameter, cell organelles sub microns and protein around 5 nm in size. Hence man made nanoparticle size are resembles to protein of living clls that facilitates the nanoparticle could enter the cell gate an acting like probes. The cellular functions may studied using nanoparticle that replaces interferon usage.[6]

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There are plenty of biological process happened in nanoscale range which paved the way to understanding and improvement of nanotechnology.[7] Nanoparticles are implemented in many therapeutic applications by desirable properties.[8]

The nanomaterials have applied various pharmaceutical purposes including diagnosing of diseases, disease prevention and treatment of diseases. The previous research discovered that metl oxide nanoparticles can kills and inhibits the growth of both gram positive and gram negative bacteria.[9]

Diabetes mellitus has been considered as a major health illness throughout the world in past decades as of now that reaches epidemic proportions.[10] Plant based drugs are best alternative treatment for diabetes instead of allopathic medicines and also side effects of herbal products are much lower than commercially available drugs.[11] There are two major reasons behinds the cause of diabetes mellitus. The first one is absence of insulin secretion and second one failure of insulin action so that diabetes commonly termed as metabolic disorders which affect 2-3% world population.[12] Recently, novel drugs are found to treat diabetes mellitus but problems will arise to utilizing of drugs on commercial purpose especially Glucosidase and lipase inhibitors have been used as antidiabetic drug in market but more side effects seen in patients who intake the medicines. For example, acarbose has suppress the blood glucose level in lower range when compared to lipase inhibitor. These lipase inhibitor drugs are causing following sickness in victims including heavy weight loss, hepatotoxicity, abdominal ache, flatulence, watery stool and hypoglycaemia.[13] Herbal plants can be used for the treatment of diabetes and devoid of side effects unlike other drugs. Plant based drugs are recommended by WHO for treating diabetes mellitus.[14] Herbal plants are safer, cost effective, unique and best effective rather than modern medicines. Among scientifically identified plants, More than eight hundred plants has antidiabetic activity according to the Indian and ayurvedic medicines. Henceforth, others are yet to be discovered.[15]

In the present study, the nanoparticle Mg doped ZnS and *Bougainvillea glabra* flower Fraction was tested against HepG2 cell lines for its antidiabetic activity.

METHODOLOGY

Preparation of Plant extract

The flowers of *Bougainvillea glabra* were collected, shade dried and finely powdered. The *Bougainvillea glabra* powder was soaked in ethanol over 24 hr and filtered through Whatman 1 paper. The ethanol was evaporated and greasy crude extract was obtained. The crude extract was dissolved in water and fractioned with chloroform and ethyl acetate. The ethanol fraction was subjected to preparative HPLC and 4 fractions were obtained. The fraction 1 was selected and used for further studies (*Bougainvillea glabra* flower fraction -BGF).

Cytotoxicity activity on hepatic cell lines

The cells were obtained from National centre for cell science, Pune, India. The cells were grown in a 96-well plate in Duelbacco Minimum Eagle's Medium (DMEM) (HiMedia) supplemented with 10% fetal bovine serum (Gibco Laboratories) and antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B). About 25 µL cell suspension (5 x 10^3 cells/well) was seeded in each 96 well and incubated at 37°C for 48 hr in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions of the samples. The cell viability was measured using MTT assay with MTT (5 mg/ml) and 10% DMSO. This tetrazolium salt is metabolically reduced by viable cells to yield a blue insoluble Formozan product measured at 570 nm spectrophotometerically.[16] Controls were maintained throughout the experiment. The assay was performed in triplicate for each of the samples. The mean of the cell viability values was compared to the control to determine the effect of the plant extracts and nanoparticles on cells and % cell viability was plotted against concentration of the samples.

Glucose uptake assay

The effects of plant extract and nanoparticle on glucose utilization were performed using HepG2 cells.[17] For this, cells were maintained at 37°C. One hundred microliters of incubation medium (8 mM glucose RPMI + 0.1% Bovine Serum Albumin (BSA)) containing specific concentration of test material was added to appropriate wells. 1 µM of insulin served as positive control. Control wells contained incubation medium only. After 1.5 h incubation, 10 µl was removed from each well and placed in to a new 96-well plate. To this, 200 µl of glucose oxidase reagent was added and incubated for 15 min before measuring absorbance at 492 nm.

Inhibition of α -Glucosidase Activity.

The α–glucosidase enzyme inhibition activity was determined by incubating 100 µl of α -glucosidase enzyme (1U/ml) solution with 100 µl of phosphate buffer (pH 7.0) which contains 100 µl of sample (0.1-100 µg/ml) or acarbose (0.1–3.2 µg/ml) at 37°C for

60 min in maltose solution. To stop the α -glucosidase action on maltose, the above reaction mixture was kept in boiling water for 2 min and cooled. To this, 2m of glucose reagent was added and its absorbance was measured at 540 nm to estimate the amount of liberated glucose by the action of α –glucosidase enzyme.[18] The percentage inhibition value was calculated.

DPPH method

The 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml of each extract were mixed with different concentration of DPPH solution followed by adding 2 ml of ethanol. The solution mixture kept at dark place for 30 min and ethanol used as blank (Figure 3). The absorbance of each sample was read at 517nm in spectrophotometer with some modification from standard methods.[19]

Statistical Analysis

The data were statistically analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) and were expressed as mean ± S.D (n = 6?). The values were considered statistically significant if the p-value was less than 0.05.

RESULTS

Table 1 described that the cytotoxicity of *Bougainvillea glabra* flower fraction (BGF), Mg doped ZnS nanoparticles against HepG2 cell lines. Liver has an important role in metabolism[20] and also maintains the glucose level in the blood by store the glucose as glycogen. More oxidative stress affects the liver cells. Hence the activity of liver is impaired and therefore it is very important that the drugs should have the protective effect on liver to maintain the normal metabolic activities.[21] Liver has significant role in metabolism and diabetes mellitus and often affected by oxidative stress.[22] In the present study, the plant fraction along with Mg doped ZnS nanoparticle showed higher hepatocyte protection at 10 µg/ml concentrations when compared with Glibenclamide.

The HepG2 cancer cell line viability were estimated by MTT assay, the drug treated cell lines has shown reduction of further growth and metabolism. The *Bougainvillea glabra* flower extract has 62.8 ± 1.8^{c} cell viability percentages which are lower than Mg doped ZnS nanoparticle (32.6± 2.5^{a}) and biosynthesized nanoparticle (37.6 ± 1.2^{b}) (Table 1) (Figure 1).

The glucose uptake activity is considered as one of the most significant test for standard antidiabetic drugs. In this plant extract exhibited 25.2 ± 1.4^{b} at 100 µg/ml and nanoparticle showed 38.6 ± 1.7^{c} concentration which lower than bio Mg doped ZnS nanoparticle (insulin used as reference compound which showed 92.6 ± 1.6^{h} (Table 2). The following three various samples of Ethanolic extracts of *Bougainvillea glabra* flower extract, Mg doped ZnS nanoparticle and bio nanoparticle were tested for α - Glucosidase inhibition activity in different concentrations. The samples were inhibited α-glucosidase with IC_{50} values of 99.9 ± 0.2^{i} , 70.7 ± 3.2^{g} and 69.8 ± 1.9^{g} µg/mL, respectively (Figure 2 and Table 3). Acarbose, used as the standard compound, showed an IC_{50} value of 30.9 ± 2.2^{cd} µg/mL. The dose-response peak of the bio Mg doped ZnS showed an high inhibition percentage at the highest concentration tested (100 µg/mL) (Table 4).

Antioxidant Profile (DPPH method)

The antioxidant activity of bio nanoparticle shows better results than plant and Mg doped ZnS nanoparticle. The plant compound exhibits 35.11% inhibition percentage at 1.0ml concentration, the same concentration nanoparticle has 21.27%. Green synthesized Mg doped Zn S nanoparticle showed best scavenging capacity of 69.4% at 1.0mg/ml concentration.

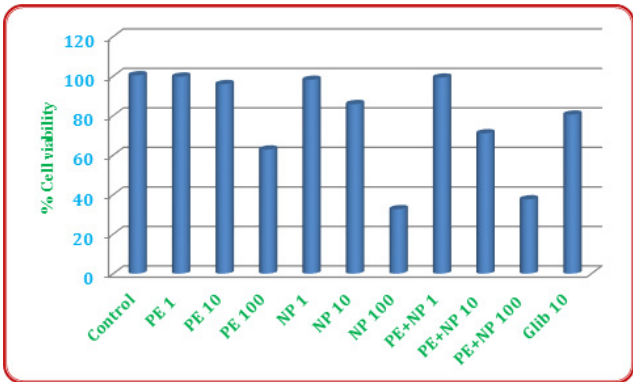

Figure 1: Estimation of cell viability against *Bougainvillea glabra* flower extract and Mg doped ZnS nano particle on HepG2 cell lines.

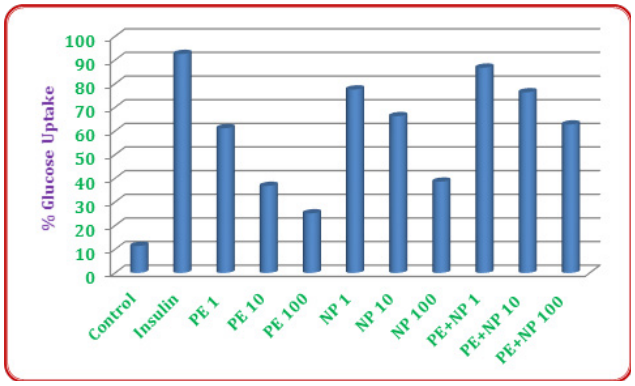

Figure 2: Glucose Uptake activity of HepG2 cell lines against *Bougainvillea glabra* flower extract and Mg doped ZnS nano particle.

Table 1: Estimation of cell viability against *Bougainvillea glabra* flower extract (Fraction) and Mg doped ZnS nano particle on HepG2 cell lines.

S. NO.	SAMPLE	CELL VIABILITY (%)
1	Control	100.4 ± 0.4^{h}
2	Plant Fraction 1 µg/ml	99.7 ± 0.6^{h}
3	Plant Fraction 10 µg/ml	95.9 ± 1.6^{g}
4	Plant Fraction 100 µg/ml	62.8 ± 1.8^{c}
5	Nanoparticle 1 µg/ml	98.1 ± 1.1^{gh}
6	Nanoparticle 10 µg/ml	71.0 ± 3.0^{d}
7	Nanoparticle 100 µg/ml	32.6 ± 2.5^{a}
8	Plant Fraction + Nanoparticle 1 µg/ml	99.2 ± 0.6^{h}
9	Plant Fraction + Nanoparticle 10 µg/ml	85.8 ± 1.5^{f}
10	Plant Fraction + Nanoparticle 100 µg/ml	37.6 ± 1.2^{b}
11	Glibenclamide 10 µg/ml	80.5 ± 2.2^{e}

Table 2: Glucose Uptake activity of HepG2 cell lines against *Bougainvillea glabra* flower extract and Mg doped ZnS nano particle.

S. NO.	Sample	Glucose Uptake
1	Control	11.4 ± 1.2^{a}
2	Insulin	92.6 ± 1.6^{h}
3	Plant Extract 1 µg/ml	61.2 ± 2.9^{d}
4	Plant Extract 10 µg/ml	36.8 ± 1.9^{c}
5	Plant Extract 100 µg/ml	25.2 ± 1.4^{b}
6	Nanoparticle 1 µg/ml	77.6 ± 1.9^{f}
7	Nanoparticle 10 µg/ml	66.3 ± 3.0^{e}
8	Nanoparticle 100 µg/ml	38.6 ± 1.7^{c}
9	Plant Extract + Nanoparticle 1 µg/ml	86.8 ± 2.5^{g}
10	Plant Extract + Nanoparticle 10 µg/ml	76.4 ± 2.8^{f}
11	Plant Extract + Nanoparticle 100 µg/ml	62.8 ± 1.9^{de}

DISCUSSION

Type 2 diabetes is often associated with magnesium (Mg) deficits.[23] Zinc supplementation in patients with diabetes had demonstrated that Zinc supplementation had beneficial effects on both glycaemic control and maintained healthy lipid parameters.[24] *Bougainvillea glabra* aqueous extract of leaves has been previously studied for its antidiabetic activity in alloxon-induced diabetic rat model.[25] The present study attempts to evaluate the possible antidiabetic activity of *Bougainvillea glabra* flower extract along with Mg doped ZnS nano particle. This study uniquely approaches to find the combination of herbal extract and nanoparticles for the effective

Table 3: α-Glucosidase inhibition activity of *Bougainvillea glabra* flower extract and Mg doped ZnS nano particle.

S. NO.	Sample	α-Glucosidase Inhibition
1	Acarbose 1 µg/ml	30.9 ± 2.2^{cd}
2	Acarbose 10 µg/ml	90.1 ± 2.0^{h}
3	Acarbose 100 µg/ml	99.9 ± 0.2^{i}
4	Plant Extract 1 µg/ml	29.6 ± 1.5^{c}
5	Plant Extract 10 µg/ml	40.2 ± 2.0^{e}
6	Plant Extract 100 µg/ml	70.7 ± 3.2^{g}
7	Nanoparticle 1 µg/ml	16.9 ± 1.5^{a}
8	Nanoparticle 10 µg/ml	22.5 ± 1.5^{b}
9	Nanoparticle 100 µg/ml	55.1 ± 1.0^{f}
10	Plant Extract + Nanoparticle 1 µg/ml	33.4 ± 2.1^{d}
11	Plant Extract + Nanoparticle 10 µg/ml	39.9 ± 3.5^{e}
12	Plant Extract + Nanoparticle 100 µg/ml	69.8 ± 1.9^{g}

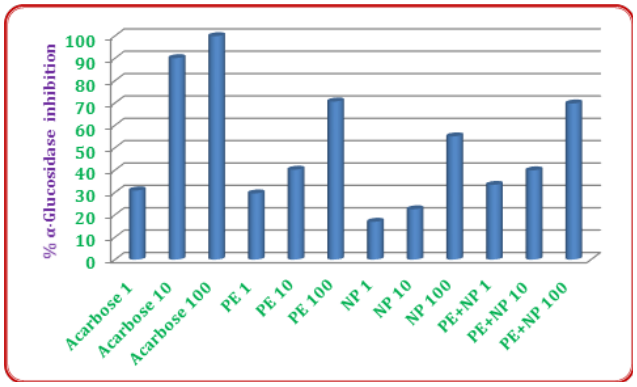

Figure 3: α - Glucosidase inhibition activity of *Bougainvillea glabra* flower extract and Mg doped ZnS nano particle.

amelioration of diabetic conditions like increased free radicals and abnormal or deficient glucose uptake. Based on the results obtained for the MTT assay, the nanoparticles synthesized and the extract alone as well as in combination did not show any significant toxic effect on the HepG2 cell lines. The glucose uptake assay and the α -Glucosidase activity inhibition assay also proved that the combination of both the extract along with Mg doped ZnS nano particles proved to be a potent antidiabetic agent. The DPPH assay also proved the *in vitro* antioxidant activity of the extract along with Mg doped ZnS nano particles. Thus, the current study provides sufficient input in verifying the antidiabetic activity of *Bougainvillea glabra* flower extract along with Mg doped ZnS nano particles.

Table 4: DPPH activity of *Bougainvillea glabra* flower extract and Mg doped ZnS nano particle.

Concentration (mg/ml)	Absorbance at 520 nm		Percentage inhibition (%)			
	A(Plant)	B(Nano particle)	Plant+NP (C) A		B	C
Control	0.94	0.94	0.59	-	-	-
0.2	0.90	0.92	0.34	4.44	0.21	42.372
0.4	0.77	0.80	0.24	18.09	14.89	59.3
0.6	0.74	0.78	0.23	21.28	17.02	61.01
0.8	0.68	0.76	0.21	27.66	19.15	64.0
1.0	0.61	0.74	0.18	35.11	21.27	69.4

CONCLUSION

This study opens up new avenues of research to further analyse the phytochemical composition of the *Bougainvillea glabra* flower extract and understand the synergic effect of the phytochemicals found in the selected fraction of the extract along with the Mg doped ZnS nano particles.

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CONFLICT OF INTEREST

The author declares no that there is no conflict of interest.

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