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RESEARCH ARTICLE

IN-VITRO ANTIDIABETIC AND ANTICANCER STUDY OF ZINC OXIDE NANOPARTICLE USING TRIPHALA - AN AYURVEDIC DRUG

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ABSTRACT

Zinc oxide nanoparticles are non-toxic, non hygroscopic polar inorganic and inexpensive material. Zinc oxide nanoparticles (ZnO) was successfully synthesized using zinc sulphate in Emblica officinalis, Terminalia chebula, Terminalia bellirica and Triphala extracts and then characterized. The antioxidant test was analyzed by Molyneux method using the strong antioxidant using DPPH method. In-vitro antidiabetic test was carried using alpha amylase and Glycation test. Alpha amylase is the breakdown of long chains of carbohydrates thus preventing the increase in blood glucose level. Glycation is the binding of sugar molecule to a protein or lipid molecules without enzymatic activity. It impairs or destroys the functioning of biomolecules. Nanoparticles annihilate cancer cells by flow and penetration to distinct regions of tumors through blood vessels and then driving to interstitial space to reach the target cells. ZnO nanoparticles exhibit highest percentage of antioxidant and in-vitro antidiabetic effect in alpha amylase and glycation test synthesized using Emblica officinalis, Terminalia chebula, Terminalia bellirica and Triphala extract. Triphala showed anticancer effect on MCF-7 cell line.

Key words: Zinc oxide nanoparticles; antimicrobial; antioxidant; antidiabetic.

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INTRODUCTION

Drugs that are used in diabetes to treat diabetes mellitus which lowers the glucose levels in blood. Alpha amylase is a protein enzyme that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. The process of conversion of starch to glucose is called as Alpha amylase. Inhibitors lowers the breakdown of the long chains of carbohydrates and prevents the increase in blood glucose level. Glycation occurs when the sugar molecule (glucose or fructose, to a protein or lipid molecule) covalently bonds without the controlling action or presence of an enzyme. Glycationoccurs inside the body or outside the body. It is a haphazard process that impairs the functioning of biomolecules, and does not require the expenditure of ATP (the energy storing molecule).Cancer is the most fatal disease cause mainly due to the genetic factor, environmental factors and also due to the rapid lifestyle changes. Nanoparticles annihilate cancer cells by flow and penetration to distinct regions of tumors through blood vessels and then driving to interstitial space to reach the target cells.According to the population surveys found that in 2014 around 29% of cancers were breast cancers, particularly found in American women (Siegel *et al*., 2014).

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MCF-7 is a breast cancer cell line isolated in 1970 from 69 years old Caucasian woman. MCF-7 is the acronym of Michigan cancer foundation.

MATERIALS AND METHODS

Antidiabetic Activity

Alpha amylase test: The effect of sample on alpha amylase activity can be studied using an enzyme - starch method. Sample was mixed with 4% potato starch, 0.1g of alpha amylase was mixed with 25mL of distilled water and stirred vigorously for 20mins and incubated at 37℃ for 60mins. After the incubation period, 0.1M NaOH was added to the solution to stop the enzyme activity. The mixture was then centrifuged at 3000rpm for 15 mins. The glucose concentration present in the supernatant was hence, measured at 546nm against reagent blank. The test was carried out at different concentration.

Glycation test: To study the effect of ZnO nanoparticles synthesised using (*Terminalia chebula, Terminalia bellirica, Emblica officinalis and Triphala*) extract by protein Glycation process. 0.075 M Phosphate buffer saline (PBS) was mixed with 25mM Glucose, 10mg/ml of sample and 1mg/ml of protein (Bovine albumin) and was incubated for 4-5 weeks at 37℃. Samples were analyzed after the incubation week i.e; after 3rd and 4th week.

Table 1. Antidiabetic activity for alpha amylase inhibition test

Concentration	*Emblica officinalis*	Triphala	*Terminalia chebula*	*Terminalia bellirica*
5 mg/mL	35.5%	39.9%	24.3%	21.3%
10 mg/mL	12.3%	61.7%	28.4%	25.5%
15 mg/mL	38.6%	87.2%	77.7%	53.1%
Control	2%	2%	4%	4%

After 4th week of incubation

Table 2. Antidiabetic Glycation test after 4th week of incubation

Sample	Glucose conc mg/ml	Protein conc mg/ml	glucose conc/mg protein conc
Triphala	1.126	0.383	2.939
Terminalia chebula	1.522	0.350	4.348
Terminalia bellirica	1.631	0.291	5.604
Emblica officinalis	2.532	0.451	5.614
Control	2.86	0.450	6.35

After 5th week of incubation

Table 3. Antidiabetic Glycation test after 5th week of incubation

Sample	Glucose conc mg/ml	Protein conc mg/ml	Glucose conc/ protein conc
Triphala	0.666	0.383	1.738
Terminalia chebula	1.465	0.350	4.185
Terminalia bellirica	1.185	0.291	4.072
Emblica officinalis	1.816	0.451	4.026
Control	2.81	0.451	6.23

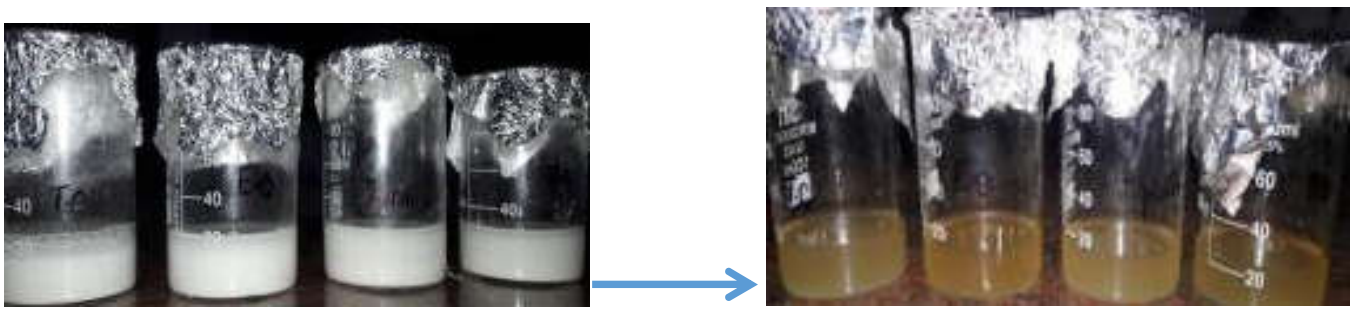
Table 4. Percentage of cytoxicity of ZnO on MCF-7 Cell line

Concentration	Percentage of cytoxicity
1ng	7.444908
10ng	10.18463
100ng	20.30971
1ug	24.06194
10ug	32.04288
100ug	37.04586
Control	3.871352

For concentration: 5mg/ml

Before adding NaOH **Colour changes to light yellow**

For concentration: 10mg/ml



Before adding NaOH **Colour changes to pale yellow**

For Concentration: 15mg/ml

Before adding NaOH

Colour changes to dark yellow

Antidiabetic assay on Glycation test

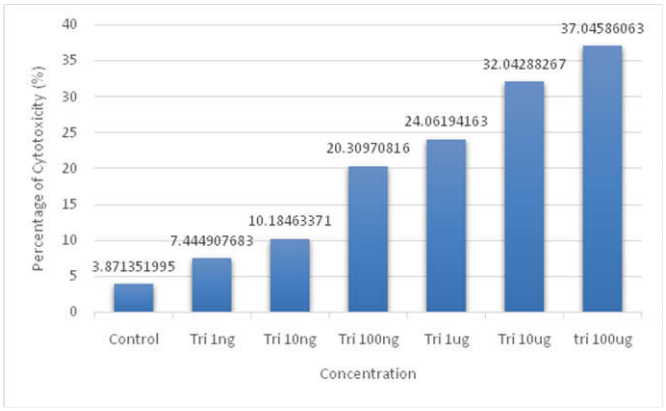
Stir the membrane loaded with sample for 2-3hrs

Adding GOD/POD and incubated at 37℃ for 10mins

Colour change occurs

Control: Triphala

ZnO: 10ug **ZnO : 1ug** **ZnO : 100ug**



Graphical representation of percentage of cytoxicity

Glucose concentration was then measured by using GOD/POD method and samples were dialyzed using a diaysis bag for removing the free glucose. Protein concentration was calculated using (Bradford Method). Dislysis was carried out for 2-3hrs and was estimated by using GOD/POD method. The sample was incubated at 37℃ for 10mins. The OD was calculated at 505nm.

Anticancer Assay: MCF 7 cell line was collected from National Centre for Cell Science, Pune. Cells was harvested in Rose well Park Memorial Institute medium (RPMI) with 10% of fetal bovine serum and 250 U/mL of penicillin or streptomycin. 100µg/mL of gentamycin and 1mg/mL of amphotericin B were collected from Sigma Chemicals, USA. All the cells grown was maintained at 37℃ at 5% CO_2. The cells were allowed to grow for achieving the confluency over 24 hours. a density of 5×10^3 cells per well for 24hours containing 200ul of RPMI dissolved in 10% FBS. The supernatant that was cultured was then removed and incubated for 48hours with different concentration of test samples containin RPMI. After the treatment, the cells were again incubated at 37℃ for 4hours containing 10µl, 5mg/mL and then with at room temperature for 1hour with DMSO. The incubated plates were then read at 595nm after the incubation using a scanning multi-well spectrophotometer.

The total percentage of cell viability can be calculated as under: Cell viability (%) = (Mean test OD/Control OD) ×100

RESULTS AND DISCUSSION

Antidiabetic Assay on Alpha amylase test: The synthesized ZnO NPs have been tested for its cytotoxic activity against MCF7 (breast cancer cells) using MTT assay. Fig. shows the cell viability calculated after 24 hour of exposure to zinc oxide nanoparticles of various concentrations ranging from (1ng/ml-100 µg/ml). Zinc oxide nanoparticles have induced cytotoxicity on MCF-7 cell line was found to be increasing with an increase in concentration of zinc oxide nanoparticles.

Conclusion: Zinc oxide nanoparticles were synthesized using zinc sulphate by using *Terminalia chebula, Terminalia bellirica, Emblica officinalis* and *Triphala* extract.In the biosynthesis of Zinc oxide nanoparticles using *Terminalia chebula, Terminlia bellirica, Emblica officinalis* and Triphala, reduces a salt to its metallic solid nanoparticles through the catalytic effect. Triphala showed highest antidiabetic activity for alpha amylase and Glycation test. This study revealed that synthesized zinc oxide nanoparticlescan be used as anticancer and antidiabetic agent.

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