

ANTI- DIABETIC AND CYTOTOXIC EFFECT OF ZINC OXIDE NANOPARTICLES SYNTHESISED USING *Boerhaavia diffusa*

RUNNING TITLE: Anti diabetic and cytotoxic effect of zinc oxide NP using boerhaavia diffusa

ABSTRACT:

INTRODUCTION: Nanotechnology is the art of manipulating matter in terms of nano scale. It mainly aims at combining nanotechnology with drugs to improve the ability of a specific target. These materials are produced at nanoscale level as it is safe to administer in the body. *Boerhaavia diffusa* is a flowering plant which is also known as Punarnava. It is a herbal medicine that is used for pain relief. It has been used primarily for its anti-diabetic and diuretic properties. Zinc oxide nanoparticles is one of the prominent metal oxide nanoparticles which is used in applications of many research projects and industries. The synthesis using plant sources is used for the production of ZnO NPs due to its environmental, economic, and medicinal benefits.

AIM: The aim of this study is to determine the anti diabetic and cytotoxic effect of zinc oxide nanoparticles synthesised using boerhaavia diffusa.

MATERIALS AND METHODS: Zinc oxide nanoparticles were synthesised using boerhaavia diffusa extract and Anti- diabetic activity was carried out using alpha amylase inhibitory activity and cytotoxic activity was done by brine shrimp lethality assay.

RESULTS: The percentage of lethality was found to be 60%, 70%, 70%, 90%, 90%, and 0% in the 5 microliters, 10microliters, 20 microliters, 40 microliters, 80 microliters and 100 microliters concentration levels. Alpha amylase inhibitory activity was performed which showed better results indicating anti diabetic and cytotoxic activity. In anti diabetic inhibitory activity the values were 40%, 50%, 75%, 80% and 82% in 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, 50mg/ml respectively. Both anti diabetic and cytotoxic effects showed prominent inhibitory effects.

CONCLUSION: Zinc oxide nanoparticles synthesised using *Boerhaavia diffusa* exhibited significant Anti -diabetic and cytotoxic activity in brine shrimp lethality assay showing emergence of a drug candidate for further research.

KEYWORDS: *Boerhaavia diffusa*, Anti- diabetic activity, Cytotoxic activity, Zinc oxide nanoparticles.

INTRODUCTION:

Nanoscience and technology deals with the science and engineering of manipulation of particles at the level of single atoms and small groups of atoms. They have a wide range in research areas, which mainly involves structures, devices, and systems(1). Nanotechnology has been used in the field of human health, which has been showing significant results, especially in the field of cancer treatment(2). Nanomaterials are the important tools of nanotechnology(3). The uniqueness of nanoparticles is mainly its size-dependent properties which makes it easier to administer in human beings(4).

Plant extracts that are biologically synthesised have a massive advantage over other biomolecules. Plant extracts have been used in the field of food, medicine, synthesis of nanoparticles and many more(5). There are many techniques developed for the production of plant extracts based on the simplicity, cost, and the type of extract content(4,6). *Boerhaavia diffusa* is a plant that belongs to the family Nyctaginaceae. It is used as traditional medicine by indigenous people of many countries because of its protective role against inflammation, diabetes, cancer, gastrointestinal problems and many more(7). The entire plant is believed to have numerous bioactive compounds which are responsible for the pharmacological activities(8). *Boerhaavia diffusa* also possesses anti aging, disease prevention, and life strengthening properties(9).

Zinc oxide nanoparticles is considered as one of the most prominent metal oxide nanoparticles because of its wide range of applications(10). It is used in applications of various research projects and industries(8). The synthesis of plant sources is used for the production of ZnO NPs due to its environmental, economic, and medicinal benefits(8,11). Zinc oxide nanoparticles are used in the production of disinfectant, anti-fungal, anticancer, antioxidant, anti-inflammatory and anti diabetic properties(12). ZnO NPs are used in biomedicine, mainly in the fields that focus on anticancer and antibacterial (13). Zinc is also known to keep the structural integrity of insulin(14). Previously our team has published extensive research on various aspects (8,15–28). This vast research experience has inspired us to do research on the anti diabetic and cytotoxic effect of zinc oxide nanoparticles synthesised using *boerhaavia diffusa*.

MATERIALS AND METHODS:

Preparation of plant extract: The fresh leaves of *Boerhavia diffusa* were collected in an unbiased manner and sampling was done by Randomised sampling method and washed thoroughly with distilled water. Only dried leaves of *Boerhavia diffusa* were included and other parts of the plant such as stem, root, flower were excluded in this study. About 1gm of clean dried leaves of *Boerhavia diffusa* was added to 50 ml of distilled water. The extract present in the conical flask is mixed well. This mixture is boiled at 60 degrees Celsius for 7 minutes with the help of a heating mantle. Then the boiled extract is filtered with the help of filter paper.

Synthesis of Zinc oxide nanoparticles: *Boerhavia diffusa* is treated with 0.507g of zinc sulphate and 90 ml of distilled water and it is placed in a semi-automatic shaker at 900 rpm. With the help of a double beam U-V spectrophotometer, the synthesis of nanoparticles for every one hour is noted. Then this formulation is placed in a centrifuge for 10 minutes. Now the synthesized nanoparticles which are settled at the bottom are collected. The randomized sampling method was done in an unbiased manner.

Anti- diabetic activity:

Antidiabetic activity Alpha mess in which the activity of behaviour decision using inculcate nanoparticles was done according to the standard method of (Ademilyi et al- 2013). In the artist you put the reaction mixture containing 500 μ l phosphate buffer (100mm, PH=6.8), 100 μ l alpha amylase(2 μ l) and varying concentration of extract (10 to 50mg/ml) was incubated at 37° for 20 minutes. The 200 μ l of 1% soluble starch(100 mm, phosphate buffer PH= 6.8) adds it as a substrate and incubates further at 37° for 30 minutes. 1000 μ l of 3,5 dinitro salicylic acid colour agent was added and boiled for 10 minutes. The absorbance of the resulting mixture was measured at 540 nanometre using a multiplate reader(multi thermo scientific, version 1.00.40). Acarbose at various concentrations(0.1-0.5 mg/ml) was used as standard. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity(%)= $[1-AS/AC] \times 100$ where,

The absorbance is in the presence of a test substance and AC is the absorbance Control. The data were subjected to statistical analysis using one way analysis of variance and Duncan's.

Cytotoxic activity:

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well(20 μ l, 40 μ l, 60 μ l, 80 μ l, 100 μ l). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24hours. After 24hours, the ELISA plates were observed and noted for the number of live nauplii present and calculated by using the following formula.

RESULTS AND DISCUSSION



FIGURE 1: Preparation of Boerhaavia diffusa extract mediated zinc oxide nanoparticles and the solution of boerhaavia diffusa in zinc sulphate solution.

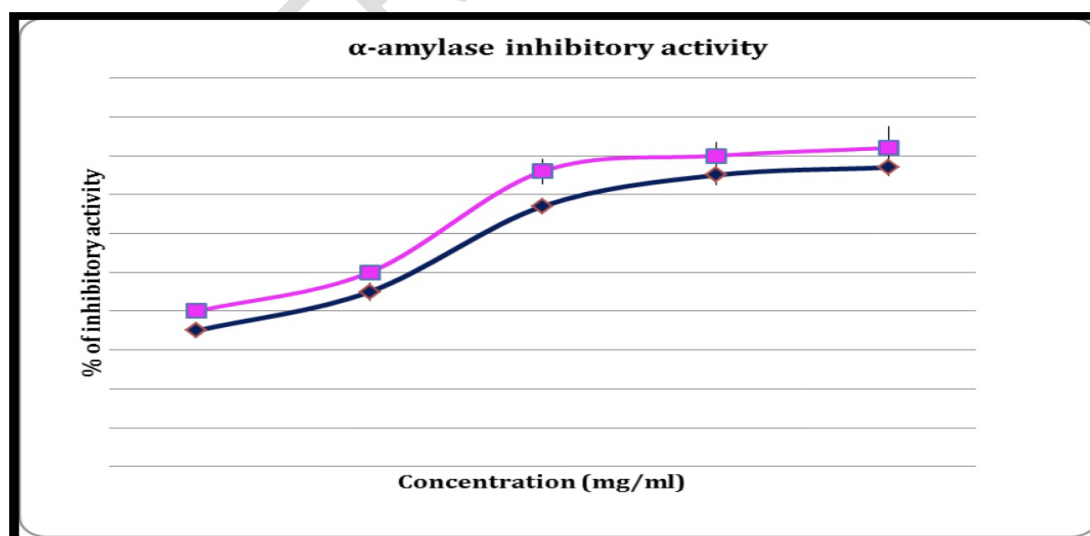


FIGURE 2: The graph representing Anti diabetic activity of Boerhaavia diffusa mediated zinc oxide nanoparticles. data implies as mean±SEM with the level of statistical significance at $p<0.05$.

The results obtained from the alpha amylase inhibitory test, the % inhibition of zinc oxide nanoparticles was consistently better than the standard drug that is acarbose. The percentage inhibition gradually increased with the increase in concentration of the drug. It also increased in the same pattern of acarbose. Zinc oxide nanoparticles are relatively better than acarbose. The results obtained from alpha glucosidase inhibitory activity, zinc oxide nanoparticles either showed the same % inhibition or slightly higher % inhibition compared to acarbose. At a concentration of 20µl and 30µl, The inhibitory action of zinc oxide nanoparticles was found to be slightly better than acarbose. Zinc oxide nanoparticles have maximum alpha amylase inhibition at 40µl and 50µl concentration. Its potential and drug action efficiency are almost similar to acarbose post which is the standard drug used as anti diabetic.

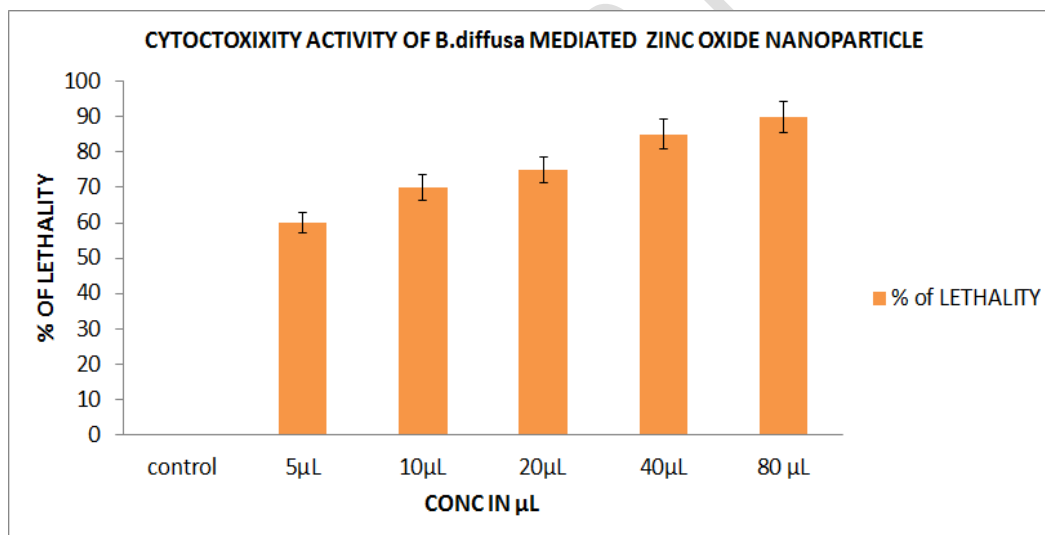


FIGURE 3: The graph presenting cytotoxic activity of Boerhaavia diffusa mediated zinc oxide nanoparticles. X- axis represents concentrations and Y- axis represents lethality, data implies as mean±SEM.

On the first day the nauplii were all alive. After 48 hours the number of nauplii changes were noted. The percentage of lethality was found to be 60%, 70%, 70%, 90%, 90%, and 0% in 5µl, 10µl, 20 µl 40µl, 80µl respectively. The maximum lethality was seen at 80 µl.

Zinc oxide nanoparticles are widely used for the production of disinfectants, anti fungal, anti cancer, anti oxidant, anti inflammatory and anti diabetic properties(29) .Previous studies show the use of plant mediated synthesis of nanoparticles which is considered as one of the most cost effective and environmental friendly methods to improve the antimicrobial activity of plant extracts(30) It is seen that biosynthesis of ZnO nanoparticles was done with the help of extraction of Boerhavia diffusa leaves and the antimicrobial activity of synthesized nanoparticles was assessed(21,31) It showed that ZnONPs could inhibit the activity of MRSA strains and could be aided as a potential antibacterial agent for cleaning and disinfection of MRSA in hospitals(23). A study by Sadhan Kumar suggests that Green synthesis of zinc oxide nanoparticles was carried out using *Calotropis* leaf extract with zinc acetate salt in the presence of 2 M NaOH, which showed that(32) .The solution of zinc oxide nanoparticles is used as fertilizer because of its ability to grow faster. Nanofertilizers are used more commonly than conventional fertilizer because of its nutrient supplies and organic state(31). A study shows that extracts of Boerhaavia diffusa leaves have been used to evaluate the antioxidant and hepatoprotective properties in the acetaminophen-induced liver damage model, which concludes that the leaf extracts of B.diffusa has exhibited hepatoprotective property against acetaminophen-induced liver damage that can be mediated through augmentation of antioxidant defenses(33). Previous article shows that biological synthesis of zinc oxide nanoparticles from the petals extract of Rosa indica L was used to determine the efficacy against two dermatophytes which concluded that ZnO-NPs acts as a potential antifungal agent for treating skin infections as it reduces the inflammation(34). An article shows that boerhaavia diffusa has shown massive therapeutic activities such as example, diuresis, anticancer, anti-inflammation, hepatoprotection, and immunomodulation (35)Although it is not that recognised in the market yet(9,34). A study confirms that the entire plant of boerhaavia diffusa contains numerous bioactive compounds which are responsible for many of its pharmacological activities. (36). A study by Pranati shows that plant extracts exhibited prominent immunomodulatory, immunosuppressive and anti-lymphoproliferative activities. Pharmacological studies on boerhaavia diffusa confirms diuretic and anti-inflammatory (35,37) activities, which makes it more suitable in the treatment of inflammatory renal diseases(38). Previously our team has published extensive research on various aspects (39)(40)(41) (42)(43)(44) (45) (46) (47)(48)(49)(50) (51)(52).

The limitations of the study is that Boerhaavia diffusa with zinc oxide nanoparticles is alone taken and silver, selenium, zinc and titanium are excluded. Other parts of boerhaavia diffusa plant such as stem, flower and roots are not studied. Further studies should involve the use of boerhaavia diffusa extract with many other nanoparticles and many other activities must be performed.

CONCLUSION:

From the above study, it can be concluded that zinc oxide nanoparticles synthesised using *Boerhaavia diffusa* shows prominent anti diabetic and cytotoxic activity in brine shrimp lethality assay showing emergence of a drug candidate for further research(53-62). It can also be used to perform antimicrobial, anti-inflammatory, antioxidant activities.

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