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## *In vitro* and *in vivo* safety evaluation of *Thespesia populnea* mediated nanoparticles

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### Abstract

The present study was conducted to evaluate the *in vitro* and *in vivo* safety of *Thespesia populnea* mediated nanoparticles. In the present study of MTT assay, the mouse splenocytes maintained a survivability of 90% at concentrations of 500 µg/ml of TPE, 42.5µg/ml of TPNS and 100 µg/ml of TPNZ respectively indicating a safe margin to use the drug at higher concentrations. In the *in vivo* safety evaluation study, oral dose of 2000, 6.8 and 80 mg/kg of TPE, TPNS and TPNZ respectively was given to mice. The acute oral toxicity test and administration of the drugs at the limit dose did not result in any mortality nor any symptoms of toxicity. There were no changes either in body weights or in necropsy findings. Hence, this dose was considered as the stopping criteria as per the guidelines and the LD50 was assumed to be more than the upper bound dose administered for each test compound.

**Keywords:** nano particles, silver, zinc, *Thespesia populnea*, *in vitro*, *in vivo*

### Introduction

Nano medicines have been an emerging therapeutic agents to meet the challenges of *S. aureus* treatment by their ability to inhibit the formation of biofilm (Park *et al.*, 2018)<sup>[11]</sup>, increased penetration of cell and biofilm membrane and enhanced intracellular retention (Yazar *et al.*, 2012)<sup>[21]</sup> and improved anti-bacterial activity of the loaded anti-microbial agents. Nano particles may offer a promising solution as they can also act as carriers for antibiotics and natural anti-microbial compounds (Wang *et al.*, 2017)<sup>[20]</sup> facilitating their intra cellular entry and sustained release of the loaded anti-microbial drugs which is useful for maintaining an optimum level of drug concentration at the site of target. Nano silver and nano ZnO are proven to have antibacterial (John, 2016 and Aparna *et al.*, 2018)<sup>[6, 1]</sup> activity against a wide range of gram positive and gram negative organisms including multidrug resistant bacteria. Further, herbal mediated synthesis of nano particles is safe to handle and possess a range of metabolites with redox potential (Kulkarni *et al.*, 2012)<sup>[8]</sup>. Phytogetic nano particles were reported to have higher antibacterial activity than nanoparticles alone (SreeVani *et al.*, 2016 and Aparna *et al.*, 2018)<sup>[17, 1]</sup>. *Thespesia populnea* commonly known as Indian tulip tree, is an evergreen tree that belongs to family, Malvaceae. The leaves of *T. populnea* contain a number of bioactive constituents like flavonoids, alkaloids, phenolic compounds, saponins and steroids (Sharma *et al.*, 2011)<sup>[14]</sup>. Further, it was reported to possess antibacterial (Krishnamoorthy *et al.*, 2014; Shekshavali and Hugar, 2012; Archana *et al.*, 2010)<sup>[7, 15, 2]</sup>, anti-inflammatory (Ilavarasan *et al.*, 2011 and Vasudevan *et al.*, 2007)<sup>[5, 19]</sup> and antioxidant activities (Vadlapudi and Naidu 2009; Raju *et al.*, 2003)<sup>[18, 12]</sup>. Both the herbal extract and nano particles together has been proved beneficial in exerting bactericidal action, reducing damage to tissues, because of their ability to penetrate deep into the cells. Hence, the present study was carried out to evaluate the *in vitro* and *in vivo* safety of *Thespesia populnea* mediated nano silver particles and nano ZnO particles.

### Materials and Methods

#### Collection and identification of plant material

The leaves of *Thespesia populnea* were collected from in and around Tirupati in Chittoor district of Andhra Pradesh. The plant was identified and authenticated by a taxonomist in the Department of Botany, University College of Science and Arts, S.V University, Tirupati.

### Preparation of methanolic leaf extract

*Thespesia populnea* methanolic leaf extract (TPE) was prepared by using cold maceration method. The leaves of *T. populnea* were shade dried and ground to a coarse powder. About 100 g of leaf powder was soaked in 500 ml of 95% methanol (v/v) for 72 h with intermittent mixing. The filtrate was concentrated by rotary evaporator and then air dried. Extract was weighed and the percentage yield was calculated with reference to the air-dried material.

### Synthesis of TPE mediated nano silver particles

*Thespesia populnea* solution (2%) was prepared by dissolving *T. populnea* methanolic leaf extract in distilled water. Silver nitrate solution (0.1M) was prepared and to 10 ml of 2% TPE, 90 ml of 0.1 M silver nitrate solution was added at 950 c with vigorous stirring. The change in colour of the solution was observed from pale yellow to brown indicating the formation of TPE mediated silver nanoparticles. The prepared solution was cooled to room temperature and particles were allowed to settle for 24 h. The solution was stored in a plastic container until further characterization.

### Synthesis of TPE mediated nano ZnO (TPNZ) particles

Around 0.25 g of zinc acetate was dissolved in 50 ml of distilled water to which 4 ml of TPE was added drop wise and the resulting mixture was stirred for 10 minutes using magnetic stirrer. Finally the pH of the solution was adjusted to 12, using NaOH. A white crystalline precipitate of zinc oxide was obtained, which was further washed repeatedly with water, filtered and dried in an oven at 60°C to obtain zinc oxide nanoparticles.

### In Vitro Evaluation of Tpe Mediated Nano Particles

#### Cytotoxicity Study (MTT Assay)<sup>[9]</sup>

##### Isolation of spleenocytes from mice

For the isolation of spleenocytes, spleen was collected from mice in a petridish with 2 ml of RPMI medium. RPMI was supplemented with 10% fetal bovine serum. The viability of cells was assessed using 0.4% trypan blue and adjusted the cell density to  $1 \times 10^7$  cells/ml using haemocytometer chamber.

##### Dilution of test compounds

Around 100 µl of the test compounds were added to the wells and made twofold serial dilution of the test compound. Further, TPE was diluted to get a series of concentrations from 2000 µg/ml to 125 µg/ml, TPNS from 170 µg/ml to 10.62 µg/ml and TPNZ from 400 µg/ml to 25 µg/ml respectively. Around 100 µl of cell suspension was added to all the wells. The ELISA plate was incubated for 16 h at 37°C in a CO<sub>2</sub> incubator. 10 µl of MTT was added to all the wells 4 h before the completion of incubation time followed by centrifugation at 1200 g for 10 minutes with discarding the supernatant. Around 100 µl of DMSO was added to dissolve the formazan formed and after 10 minutes the absorbance was read at 530 nm in an ELISA reader.

### In Vivo Safety Evaluation

#### Acute oral toxicity testing<sup>[10]</sup>

##### Procedure

Acute oral toxicity test was conducted by Up and Down procedure as described by OECD guidelines 425. Healthy adult Swiss albino female mice of 8-12 weeks of age and weighing 25-35 g were used for the present study. The animals were caged in a solid bottom polypropylene cage and

were managed as per OECD guidelines. The mice were kept fasting for overnight prior to dosing of the test compound. The maximum recommended oral dose volume of 20 ml/kg body weight, i.e., 2,000 mg/kg.b.wt. of TPE, 6.8 mg/kg.b.wt. of TPNS and 80 mg/kg b. wt. of TPNZ were taken as the upper bound dose.

### Results and Discussion

#### Cytotoxicity Study (MTT Assay)

Safety evaluation of TPE, TPNS and TPNZ was carried out by MTT assay *in vitro* in mouse spleenocytes and limit oral acute toxicity was carried out *in vivo* in mice. In the MTT assay, the mouse spleenocytes maintained a survivability of above 80% at all the concentrations of TPE, TPNS and TPNZ except at 400 µg/ml conc. of TPNZ.

In the present study of MTT assay, the mouse spleenocytes maintained a survivability of 90% at concentrations of 500 µg/ml of TPE, 42.5 µg/ml of TPNS and 100 µg/ml of TPNZ respectively indicating a safe margin to use the drug at higher concentrations. Somayyeh *et al.* (2011)<sup>[16]</sup> conducted a toxicity study of chemical mediated nano silver on osteoblast cancer cell lines and demonstrated concentration dependent toxicity and IC<sub>50</sub> value of 3.42 µg/ml in that cell line. Faedmaleki *et al.* (2012)<sup>[4]</sup> reported the cytotoxic effect of chemical mediated silver nano particles in Hep G2 cell line and mice liver primary cell culture and found IC<sub>50</sub> value of 2.76 ppm and 121.7 ppm respectively. Aparna *et al.* (2018)<sup>[2]</sup> reported no toxicity of mouse spleenocytes up to 85 ppm concentration of *B. ovalifoliolata* mediated AgNPs. Shaalan *et al.* (2017)<sup>[13]</sup> reported that AgNPs are less cytotoxic than ZnO NPs based on the observations of MTT assay in Eel kidney cells

#### In vivo safety evaluation

In the safety evaluation study, oral dose of 2000, 6.8 and 80 mg/kg of TPE, TPNS and TPNZ respectively was given to mice. The acute oral toxicity test and administration of the drugs at the limit dose did not result in any mortality nor any symptoms of toxicity. There were no changes either in body weights or in necropsy findings. Hence, this dose was considered as the stopping criteria as per the guidelines and the LD<sub>50</sub> was assumed to be more than the upper bound dose administered for each test compound. The oral toxicity dose currently used was many times higher than the therapeutic dose used in the experimental study.

In the safety evaluation study, no mortality was found even up to 2 weeks. This indicates that the LD<sub>50</sub> doses will be much higher for these compounds, hence these compounds can be safely used at the doses specified. Similar results were reported by Chaitanya *et al.* (2013)<sup>[3]</sup> during safety evaluation of aloin mediated nano silver in rats an observed that an upper bound dose of 3.5 mg/kg body weight didnot produce any toxicity.

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