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RESEARCH ARTICLE

ASSESSMENT OF TOXICITY STUDY OF ETHANOLIC EXTRACT AND SYNTHESIZED SILVER NANOPARTICLES OF *MORINGA CONCANENSIS* NIMMO LEAVES USING WISTER ALBINO RATS

***Malathi, R., Chandrasekar, S. and Sivakumar D.**

Research Department of Biotechnology, Bharathidasan University Constituent College, Kurumbalur, Perambalur – 621107, Tamilnadu, India

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ABSTRACT

Background and Objective: Here we are planning to study the toxicity of the ethanolic extract of *M.concanensis* leaves and synthesized silver nanoparticles from the aqueous extract of *M.concanensis* leaves by using wister albino rats.
Material and Methods: The green route method was employed to synthesize the silver nanoparticles from aqueous extract of *M.concanensis* leaves. The toxicity study of *M.concanensis* leaves was studied by observing the changes on hematological measurements such as hemoglobin, PCV, WBC, RBC and Platelets.
Results: Among all the tested extracts of *M.concanensis* Nimmo leaves, chloroform, ethyl acetate and aqueous extracts has the lowest number of phytochemicals. The ethanolic extract of the leaves was found rich source of phytochemicals as compared to the other extracts. The aqueous extract of *Moringa concanensis* Nimmo leaves revealed that it has a very good source of silver nanoparticles. Based on the study the 200mg of ethanlic extract and 150µg of silver nanoparticles was selected as an optimum dose.
Conclusion: Based on the above findings, it was concluded as this research work may gives a clear understand on the toxicity of ethanolic extract and silver nanoparticles from the leaves of *Moringa concanensis* Nimmo. This results may used in the further research in this plant.

Key words: *M.concanensis* leaves, Silver nanoparticles, Toxicity, Phyhtochemical analysis and Wister rats.

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INTRODUCTION

Pharmacological evaluation of medicinal plants has recently witnessed a growing interest amongst researchers worldwide. Research on the therapeutic potential of plants has surged over the years, with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases (Benzie *et al.,* 2011). However, while voluminous pharmacological studies have been conducted to ascertain the subjective traditional uses of various medicinal plants, very few plants have been thoroughly evaluated for their detrimental effect. Reports of efficacy are, by far, more numerous than those on toxicity (Ekor, 2014; Chalut, 1999). There is, therefore, a need to further the investigation of herbal remedies and phytochemicals to incorporate the observations of short and long-term toxicity manifestations and to ensure effectual open communication of such findings.

Corresponding author: **Malathi, R.,**
Research Department of Biotechnology, Bharathidasan University Constituent College, Kurumbalur, Perambalur – 621107, Tamilnadu, India.

Some medicinal plants that were once considered non-toxic have been reported to be hepatotoxic while some have been reported to be responsible for renal impairment (D'Arcy, 1991; Shafaei, 2011). Therefore proper and detailed toxicological assessment should form a critical component of both early and late phases of drug development from medicinal plants to avoid toxicity tragedies after such drugs might have been approved for therapeutic purposes. One way to determine the toxicity profile of herbal preparations is to assess their effects on hematological and biochemical parameters (Aboderin, 2006). The present study deals with the dose fixation of the synthesized silver nanoparticles from aqueous extract of *M.concanensis* leaves and ethanolic extract of *Moringa concanensis* Nimmo leaves in experimental animals.

MATERIALS AND METHODS

Collection and identification of plant

The healthy, matured and insect bites free leaves of *Moringa concanensis* Nimmo plant (Family - *Moringaceae*) was

collected from Esanai village, Perambalur district, Tamilnadu, India (Latitude – 11.2982° N, Longitude – 78.8298° E). The plant sample was identified and authenticated by Dr. C. Murugan, Scientist, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification number BSI/SRC/5/23/2016/Tech-152.

Preparation of plant extracts

The *Moringa concanensis* Nimmo leaves were washed, shade dried and powdered using mixer grinder. The powdered leaves (10 gms) was extracted with 100 ml of selected organic solvents (aqueous, methanol, ethanol, chloroform and ethyl acetate) using soxhlet apparatus. The concentrated solvents extract of the leaves was stored in refrigerator for further analysis.

Synthesis of silver nanoparticles

Preparation of plant extract

Aqueous extract of *Moringa concanensis* Nimmo leaves was used to synthesize the silver nanoparticles on the basis of cost effectiveness, ecofriendly sources, easy available and its medicinal values. The 10 gms of the dried *M.concanensis* Nimmo leaves powder were kept in a beaker containing 100 ml double distilled water and boiled at 80˚C for 10 minutes to obtained bioactive compounds from *Moringa concanensis* Nimmo leaves. The extract was cooled and filtered through normal filter paper followed by Whatmann filter paper No.1. The final extract was used to synthesis silver nanoparticles.

Synthesis of silver nanoparticles

The aqueous solution of silver nitrate ($AgNO_3$) at concentration of 0.001 M was prepared to synthesize silver nanoparticles from filtered aqueous extract of *Moringa concanensis* Nimmo leaves. The 5 ml of aqueous extract *Moringa concanensis* Nimmo leaves was slowly added to 95 ml of aqueous solution of $AgNO_3$ while stirring for reduction into Ag ions. The formation reddish brown colour was observed after 3hrs incubation at room temperature the AgNPs solution was purified by repeated centrifugation at 10,000 rpm for 20 minutes to isolate AgNPs free from other bioorganic compounds present in the reaction medium. After centrifugation the obtained particles were washed with distilled water for 10 to 20 minutes and kept in hot air oven for drying at 100˚C for 1 hour.

Characterization of silver nanoparticles

UV-Visible spectroscopy analysis: The optical measurement is the prime technique for characterizing the biological synthesis of nanoparticles. The formation and stability of silver nanoparticles in aqueous solution was confirmed by UV-Visible spectrophotometer analysis. The reduction of pure Ag^+ ions was monitored by measuring the UV-Visible spectrum of the reaction medium at 540 nm for 12 hours. UV-Visible spectral analysis was done by UV-Visible spectrophotometer (UV- 2450, Shimadzu).

Scanning electron microscope (SEM): Scanning electron microscope (SEM) analysis was done using Hitachi S – 4500 SEM machine. The silver nanoparticles were centrifuged at 10,000 rpm for 30 minutes and the pellet was redispersed in 10 ml of ethanol and washed 3 times with sterile distilled water to obtain the pellet. The pellet was dried in an oven and thin films of dried samples (10 mg / ml) were prepared on carbon coated copper grid and analysed for size determination. The particle size and texture of nanoparticles can be analysed by using image magnification software compatible with SEM and helps in determining the presence and formation of silver nanoparticles.

Fourier transform – Infra red analysis (FT-IR): The FT-IR spectroscopy measurements are carried out to identify the biomolecules that bound specifically on the silver surface and local molecular environment of capping agent on the nanoparticles. To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 10,000 rpm for 10 mins and the resulting suspension was redispersed in 10 ml sterile distilled water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analysed by FT-IR spectrophotometer.

Preliminary animal studies in toxicity studies of dose fixing for ethanolic extract of *Moringa concanensis* Nimmo leaves

Selection of animals: Healthy adult male wistar albino rats weighing about 150 to 200 gms were obtained from Viprogen Bioscience Private Limited, Mysore. They were housed in polypropylene cages under the standard laboratory condition (25 ± 2˚C, humidity 60-70%, 12 hours light/dark cycles). The rats were fed with commercial rat pellet diet and water was provided ad libitum. The rats were acclimatized to laboratory conditions for one week prior to the commencement of the experiments. The animal care and handling were done according to the regulations of council directive of committee for the purpose of control and supervision of experiments on animals (CPCSEA) on animal experimentations. The clearance No: VIP/IAEC/8/2016). All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the International Animal Ethics Committee (IAEC).

Experimental design

Group I: Normal rats (Standard diet)
Group II: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 100 mg/kg body weight for 14 days.
Group III: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 200 mg/kg body weight for 14 days.
Group IV: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 400 mg/kg body weight for 14 days.
Group V: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 800 mg/kg body weight for 14 days.

Preliminary animal studies in toxicity studies of dose fixing for silver nanoparticles

Experimental design

Group I: Normal rats (Standard diet)
Group II: Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo 50 µg/kg body weight for 14 days.

Group III: Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo100 µg/kg body weight for 14 days.

Group IV: Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo 150 µg/kg body weight for 14 days.

Group V: Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo 200 µg/kg body weight for 14 days.

They were continuously observed for 4 hrs to detect any changes in the behavior in relation to the posture, mood and motor activity.

Collection of samples

After the experimental regimen (4 weeks), the rats were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected in EDTA coated centrifuge tubes by an incision made in the jugular veins and serum was separated by centrifugation at 2000 rpm for 20 minutes and utilized for various biochemical assays.

Hematological parameters

The haematological parameters such as haemoglobin, PCV, WBC, RBC and Platelets were assayed. The whole blood sample was analysed for the changes in the blood cells using SYSMEX Xs – 800 i automatic haematology analyzer.

RESULTS

Biosynthesis of silver nanoparticles from *Moringa concanensis* Nimmo leaves

Visual observation

The silver nanoparticles were synthesized from aqueous extract of *Moringa concanensis* Nimmo leaves. The formation of silver nanoparticles from reaction medium was confirmed by colour change. The reaction mixture contains the aqueous extract of *M.concanensis* Nimmo leaves and aqueous silver nitrate solution. After the 24 hrs of dark incubation the colour of the reaction medium was changed from light brown to black (Figure 1).

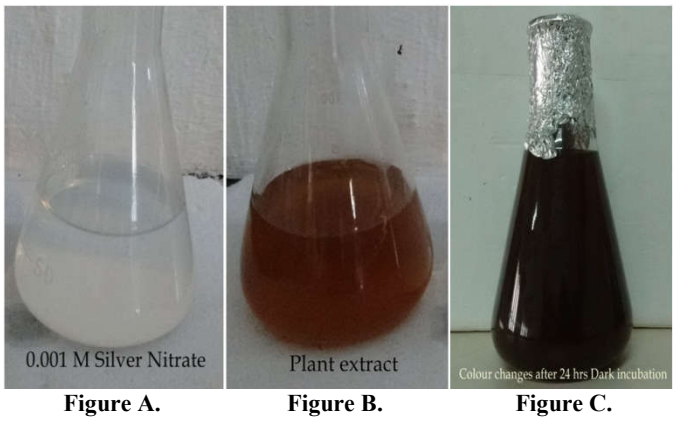

Figure A. **Figure B.** **Figure C.**

Figure 1. Synthesis of silver nanoparticles from *M.concanensis* Nimmo leaves

Figure A – Aqueous silver nitrate solution (0.001M)
Figure B – Aqueous extract of *Moringa concanensis* Nimmo leaves
Figure C – Reaction medium after 24hrs dark incubation

UV-Visible Spectroscopy analysis of synthesized silver nanoparticles from aqueous extract of *M.concanensis* Nimmo leaves

The UV spectrum showed (Figure 2) the surface plasma AgNPs at increasing concentration was taken and the colour changes were observed for nanoparticles. For silver colour changes from colourless to dark brown colour. Metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules. In green synthesis, this is observed that natural material extract act as reducing agent for generation of metal nanoparticles**.**

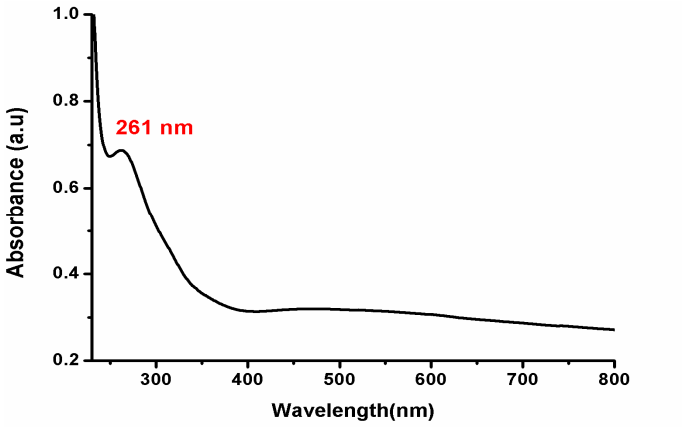

Figure 2. UV visible spectral analysis of silver nanoparticles

The figure showing the UV–Visible spectrum of silver nanoparticles synthesized from aqueous extract of *Moringa concanensis* Nimmo and absorbance peak noted at 261 nm.

Scanning electron microscopic analysis of synthesized silver nanoparticles from aqueous extract of *M.concanensis* Nimmo leaves

The SEM image showed individual silver nanoparticles as well as a number of aggregates, SEM images of silver nanoparticles derived from the leaf extracts of *M.concanensis* Nimmo showed particles to be in spherical shape with size ranging from 0.2 to 1 µm (Figure 3).

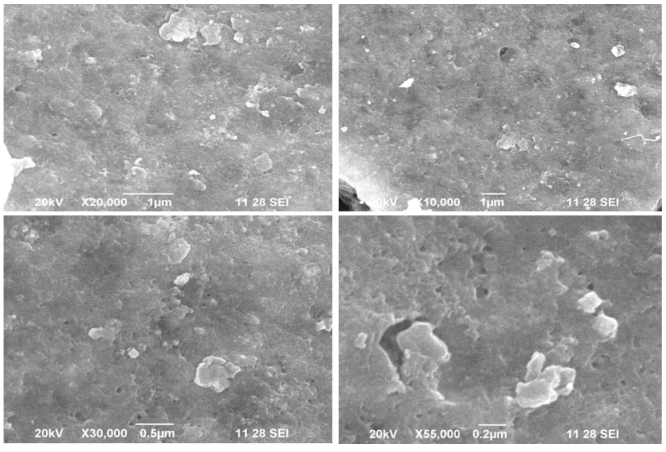

Figure 3. Scanning electron microscopic analysis silver nanoparticles

The figure showing the scanning electron microscopy (SEM) micrographs of synthesized silver nanoparticles from *Moringa concanensis* Nimmo leaves (MCAgNPs), showing the morphology of nanoparticles.

Table 1. Effect of ethanolic extract of *Moringa concanensis* Nimmo leaves on hematological parameters

Experimental groups	Hb (g %)	PCV (%)	WBC (10^3 /µl)	RBC (10^{12}/ µl)	Platelets (10^9 /µl)
Group I (Control)	12.6 ± 0.30	37.50 ± 0.81	6.50 ± 0.40	5.80 ± 0.30	5.24 ± 0.30
Group II (100 mg / kg b.w)	12.6 ± 0.50	37.50 ± 1.10	6.20 ± 0.30	5.70 ± 0.50	5.20 ± 0.40
Group III (200 mg / kg b.w)	12.6 ± 0.10	37.50 ± 0.50	6.40 ± 0.40	5.80 ± 0.50	5.25 ± 0.30
Group IV (400 mg / kg b.w)	12.1 ± 0.15	36.12 ± 0.60	5.23 ± 0.10	5.10 ± 0.50	5.12 ± 0.30
Group V (800 mg / kg b.w)	11.8 ± 0.20	36.05 ± 1.20	5.08 ± 0.30	5.15 ± 0.40	4.96 ± 0.20

Values are expressed as mean ± SD of six animals in each group ($p < 0.05$)

Table 2. Effect of silver nanoparticles of *Moringa concanensis* Nimmo leaves on hematological parameters

Experimental groups	Hb (g %)	PCV (%)	WBC (10^3 /µl)	RBC (10^{12}/ µl)	Platelets (10^9 /µl)
Group I Control)	12.6 ± 0.30	37.50 ± 0.81	6.50 ± 0.40	5.80 ± 0.30	5.24 ± 0.30
Group II (50 µg / kg b.w)	12.6 ± 0.20	37.50 ± 0.60	6.40 ± 0.50	5.75 ± 0.40	5.23 ± 0.50
Group III (100 µg / kg b.w)	12.5 ± 0.30	37.50 ± 0.50	6.50 ± 0.40	5.80 ± 0.30	5.24 ± 0.30
Group IV (150 µg / kg b.w)	12.6 ± 0.10	37.50 ± 0.40	6.50 ± 0.50	5.80 ± 0.31	5.25 ± 0.20
Group V (200 µg / kg b.w)	12.1 ± 0.30	37.00 ± 0.30	6.10 ± 0.30	5.20 ± 0.40	4.90 ± 0.40

Values are expressed as mean ± SD of six animals in each group ($p<0.05$)

Animal studies – Toxicity studies for dose fixing

Effect of ethanolic extract of *Moringa concanensis* leaves on hematological parameters in blood of experimental rats: The dose fixation study was employed in various concentration of EEMC i.e., 100 mg/ kg of body weight to 600 mg/kg of body weight. The obtained result (Table 1) showed that there was a no significant ($p<0.05$) changes in hemoglobin, packed cell volume (PCV), white blood cells (WBCs), red blood cells (RBCs) and platelets with EEMC up to 200 mg/ kg of body weight in very close relation with control group. However 400 mg/ kg of body weight and 600 mg/ kg of body weight of EEMC treated rats showed a significant ($p<0.05$) decrease in relative blood components. The significant ($p<0.05$) decreases in these blood components may due to the higher doses of ethanolic extracts of *Moringa concanensis* Nimmo leaves.

Effect of Silver nanoparticles of *Moringa concanensis* leaves on hematological parameters in blood of experimental rats: The dose fixation study was employed in various concentration of silver nanoparticles i.e., 50 µg/ kg b. w to 200 µg/ kg of body weight. The table 2 showed that there was a no significant ($p<0.05$) changes in hemoglobin, packed cell volume (PCV), white blood cells (WBCs), red blood cells (RBCs) and platelets with EEMC up to 150 µg/ kg of body weight in relation with control group. However 200 µg/ kg b.w of silver nanoparticles treated rats showed a significant ($p<0.05$) decrease in relative blood components. The significant ($p<0.05$) decreases in these blood components may due to the higher doses of silver nanoparticles.

DISCUSSION

For centuries, natural products, such as medicinal plants have been the basis for the treatment of various ailments (Ridtitid *et al.,* 2008). In screening natural products for the pharmacological activity, assessment and evaluation of the toxic characteristics of a natural product extract, fraction, or compound are usually an initial step. Regardless of the pharmacological beneficial effects of M.concanensis *Nimmo*, detailed knowledge about the chronic toxicology of this famous herb is lacking. Hence, the current study was undertaken to evaluate and focus on the acute and chronic toxicity of M.concanensis *Nimmo* in an animal model. After the 24 hrs of dark incubation of aqueous extract with silver nitrate solution, the colour of the reaction medium was changed from light brown to black (Figure 1).

This result indicates the formation of silver nanoparticles by the reduction of Ag ions. Similar results were observed in various plants studied by Aravinthan *et al.,* (2015). The UV–Visible spectroscopy was an important technique for analyzing the formation of silver nanoparticles (AgNPs) in the reaction medium. Aqueous solution of AgNPs has free electron, which gives rise to plasma resonance absorption band, due to combined vibration of metal nanoparticles in resonance with the light wave. A surface plasma resonance spectrum of AgNPs was obtained at 261 nm after 5 min colour changing to light yellowish colour. This may be due to the excitation of Surface Plasmon Resonance (SPR) of the synthesized Ag NPs (Rajakumar and Rahuman, 2011). The SPR band at 410 to 430 nm confirmed the synthesis of AgNPs at plant products (Sathishkumar *et al.,* 2009). Similar results were obtained from silver nanoparticles synthesized by using the leaf extract of *Tylophora indica* Merr plant by the method of reduction. This reduction of silver chloride to nano silver resulted in colour change (Thombre *et al.,* 2015). Similar results were observed by Rastogi and Arunachalam (2011) for the SNPs synthesized using the aqueous garlic extract under sunlight irradiation and by Suman *et al.,* (2013) for the SNPs synthesized using the root extract of *Morinda citrifolia*.

UV-Vis spectrophotometer allows identification, characterization and analysis of metallic nanoparticles. In general 200-800 nm light wavelength was used for the characterization of size range 2-100 nm (Feldheim and Foss, 2002). The morphology of the silver nanoparticles was predominantly spherical and they appear to be monodispersed. Further, analysis of the silver particles by energy dispersive spectroscopy confirmed the presence of the signal characteristic of silver. In SEM analysis, there were observed few traces of AgNPs clusters due to aggregation of nanoparticles (Figure 3), which might be induced by the evaporation solvent during sample preparation (Suresh *et al.,* 2014). The result was comparable with the *A.nilagirica* leaf extract mediated silver nanoparticles by Vijayakumar *et al.,* (2013). The scanning electron microscopy (SEM) is a common method for surface and morphological characterization. Scanning electron microscopy (SEM) is used for the morphological characterization at the nanometer to micrometer scale (Schaffer *et al.,* 2009). Ali *et al.,* (2011) reported that the studies of characterization by scanning electron microscopy have provided more information on the synthesized silver nanoparticles in the presence of papaya leaf extract such as size and morphology of nanostructures at scale of 200 nm

showed the nanoparticles has 1 µm and 2 µm size. Balaji Venkatesan *et al.,* (2014) reported that the scanning electron microscopic analysis provides a clear idea on the shape of nanoparticles isolated from aqueous extract of *Rosa damascena*. Majority of the particles were spherical in shape with the size ranging from 15 to 27 nm. These particles were well distributed without any aggregation. For the dose fixation studies the ethanolic extract of *Moringa concanensis* Nimmo leaves (EEMC) was treated with the various concentrations in the rats and the hematological parameters in the blood of experimental rats was analyzed. When compare with control rats (Group I), similar changes in the relative blood components of experimental rats treated with 100 mg/ kg, 200 mg/ kg, 400 mg/ kg and 800 mg/ kg EEMC for 14 days was showed in the table 1. Here the present study revealed that the, 200 mg/ kg of dose did not showed any harmful effects in treated rats but there was a significant ($p<0.05$) decrease in the blood components of experimental rats treated with EEMC 400 mg/ kg of body weight and 600 mg/kg of body weight as compared with control rats. This result clearly indicates that the 200 mg/kg per body weight was the optimum dose for the further investigation. For the dose fixation studies the synthesized silver nanoparticles from aqueous leaves extract of *Moringa concanensis* Nimmo was treated with the various concentrations and then the hematological parameters in the blood of experimental rats was analyzed. When compare with control group, similar changes in the relative blood components of experimental rats treated with silver nanoparticles for 14 days were showed in the table 2. Here, a significant ($p<0.05$) decrease in the blood components of experimental rats treated with silver nanoparticles (200 µg/ kg b.w) as compared with control rats. The treatment with the silver nanoparticles at the concentration of 150 µg/ kg body weight showed the significantly ($p<0.05$) similar results when compared with control rats. This result clearly indicates that the treatment with the 150 µg/ kg b.w showed the no any effective changes on hematological components of experimental rats. Hence the dose of 150 µg/ kg b.w was selected as optimum dose for the further studies.

Conclusion

The above mentioned results clearly indicates that the 200mg of ethanloic extract of *Moringa concanensis* Nimmo leaves and 150µg of synthesized silver nanoparticles should not possesses any toxic effects of hematological parameters of experimental rats. Hence it was concluded that the 200mg of ethanolic extract of leaves and 150µg of synthesized silver nanoparticles were the optimum dose for the future studies on this plant.

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