



PREVENTION OF ZNO NANO-PARTICLES INDUCED TOXICITIES BY MORINGA OLIEFERA SEED EXTRACT IN SWISS ALBINO MICE

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ABSTRACT

AIM: The present investigations were undertaken to evaluate prevention of toxicity with moringa oliefera treatment in ZnO nano particles induced toxicities in Swiss albino mice.

Methods: The effects of orally administered moringa oliefera on serum creatinine, SGPT and CK-MB expressions were observed in ZnO nano toxicities induced mice and treated groups, over a period of 21 days treatment.

Results: ZnO nano toxicities induced groups produced a significant increased serum creatinine, SGPT and CK-MB expressions and treatment with Moringa oliefera, significantly ($p < 0.05$) decreased serum creatinine levels, SGPT levels and CK-MB expressions and body weight., because the administration of Moringa oliefera to ZnO nano toxic groups, reduced the effect of ZnO nano toxicities.

Conclusion: Our results suggesting that Moringa oliefera drug therapy to be beneficial for the treatment of ZnO nano toxicities in mice.

Key words: Moringa oliefera , ZnO nano toxicity, Serum Creatinin, CK-MB expressions.

INTRODUCTION

Nanoparticles (NP) are defined as particles with a diameter smaller than 100 nm, are increasingly used in different applications, including drug carrier systems and to pass organ barriers such as the blood-brain barrier. Because of their unique properties of Nanocrystals (quantum dots) and other nanoparticles (gold colloids, nanobars, dendrimers and nanoshells) have been receiving a lot of attention for potential use of therapeutics bioengineering and therapeutics drug discovery. (Abhilash et al., 2010). Nanomaterials such as enhanced magnetic, catalytic, optical, electrical, and mechanical properties when compared to conventional formulations of the same material (Ferrari, 2005). Zinc oxide (ZnO) is an important material for nanotechnology and is increasingly employed in commercial products (Nohynek et al., 2007). The most visible applications for zinc oxide are in skin care for example sun screens, ointments, creams, powders and UV protection visible textiles (Kathrivelu et al., 2009). Large amounts of ROS could be generated even when only small amounts of ZnO and other NPs are incorporated into cells (Kasemets et al., 2009).

Moringa oleifera Lam. is a drumstick tree based on the appearance of its immature seed pods, the horseradish tree based on the taste of ground root preparations, and the ben oil tree from seed-derived oils. In some areas, immature seed pods are eaten, while the leaves are widely used as a basic food because of their high nutrition content (Razis et al., 2014).

M. oleifera Seeds, leaves, oil, sap, bark, roots, and flowers are widely used in traditional medicine. *Moringa* leaves have been characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids, and fatty acids (Moyo et al., 2011; Razis et al., 2014). The leaves of *M. oleifera* having antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani et al., 2013; Vongsak et al., 2014). *M. oleifera* is used for anti-inflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, cardioprotective and hepatoprotectant activities (Vongsak et al., 2014). *Moringa oleifera* leaves are found to be a potential source of natural antioxidants and are therefore reported to possess antinephrotoxic effects (Fakurazi et al., 2012). The therapeutic effects of *M. oleifera* include anti-hepatotoxic effect as well. In spite of having a toxic effect on the liver by itself, which is seen as an increase in the plasma AST and ALT levels (Vinodini et al., 2014; Kasolo et al., 2012).

MATERIALS AND METHOD:

ZnO nano particle powder (nanorods) was procured from "International Advanced Research center for Powder Metallurgy and New materials", Hyderabad. The method by which ZnO Nanorods were prepared by flame spray pyrolysis (FSP) and is pre-characterized and the characterization details are as follows.

Animals: The male albino mice weighing 20-25g were procured from Sainath agencies, Hyderabad. Mice were housed and cared for under pathogen free conditions. Mice were given standard diet and water ad libitum and were housed in a 12h light/dark cycle. In addition mice were acclimatized for 1 week to laboratory environment prior to study. From this dispersion, which serves as a stock, and from which the required doses were administered.

Experimental procedure:

Swiss Albino mice were divided into 5 groups each containing 6 animals.

Group I serves as a normal control

Group II and III serve as toxic groups (ZnO NPs 200 & 400 mg/kg, with 1% Sod.CMC *p.o.*).

Group IV and V serve as preventive groups (Moringa Oleifera 250 & 500 mg/kg, with 1% Sod.CMC *p.o.*).

Determination of serum creatinine (Scr): Serum creatinine was determined by *Jaffe's Alkaline Picrate* method (Taussky *et al.*, 1956). In alkaline medium picric acid reacts with creatinine and produces a red colored complex, whose absorbance is proportional to creatinine concentration. Picric acid reagent has a dual role, as a deproteinizing agent and as a reactant.

Measurement of serum indices of cardiotoxicity: Serum creatine phosphokinase isoenzyme CK-MB activity was measured kinetically at 340 nm according to standard methods using diagnostic kits from (Stanbio laboratory, INC. USA). Serum aspartate aminotransferase (AST) activity was determined according to the method of (Collinson *et al.*, 1998)

Estimation of SGPT (ALT) & SGOT (AST): Estimation of SGOT (AST): Aspartate aminotransferase (AST) also known as glutamate oxaloacetate transaminase (GOT) is a transaminase. AST catalyses the transfer of the aminogroup of L-aspartate to a ketoglutarate to give L-glutamate. AST is widely distributed in the body, but the highest levels are found in heart, liver, skeletal muscles and kidneys (Cohen *et al.*, 1979).

RESULTS

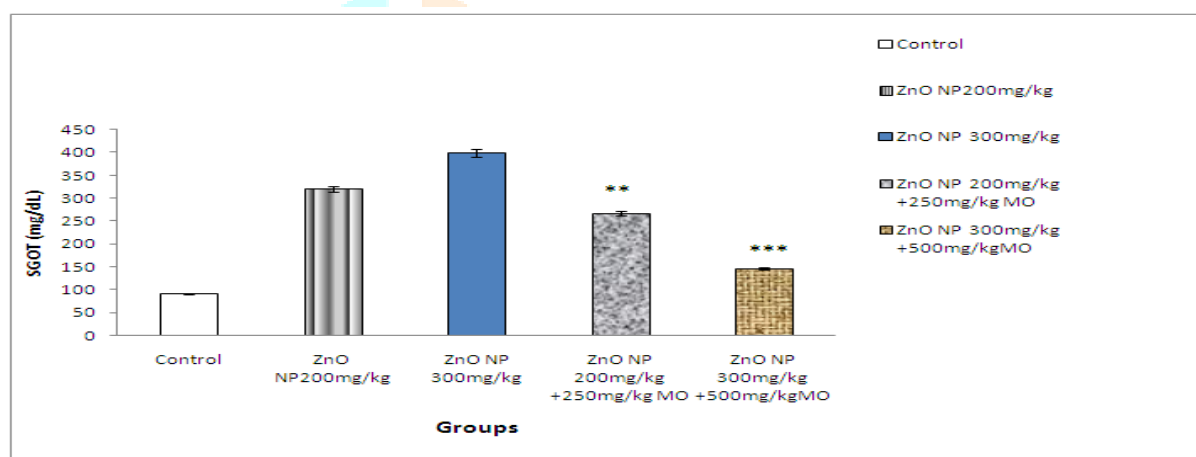
The serum creatinine levels were elevated in toxic groups (ZnO NP, 200 mg/kg and 400 mg/kg groups) after inducing zinc oxide NP. Whereas in the treatment group with Moringa oleifera the levels were found that decreased when compared to the induced groups. The SGPT and SGOT levels were elevated in 200 mg/Kg and 400 mg/kg groups. After the preventive therapy with Moringa oleifera, the SGPT and SGOT levels were found that decrease when compared with the induced groups. The CK-MB levels were expressed more in 200 mg/Kg and 400 mg/kg ZnO NP groups. After the treatment with Moringa oleifera the levels were found to be decrease when compared with the induced groups. The control group animals were showed that there was no significant change in body weight. Whereas in the other preventive groups the moringa oleifera there was a significant change in the body weight. The body weights were found to be increased in the preventive group, and the ZnO NP treated groups lost their weight. These results of Serum creatinine, weight, CKMB, SGOT & SGPT were expressed in table.1, Fig 1&2 and histological changes were decreased in liver, kidney heart tissues are represented in Fig.3.

Table 1: Effect of Moringa oleifera on ZnO NPs induced Serum Creatinine, weight and CKMB levels in swiss albino mice (Mean±SD):

| Parameters/ Groups | Serum creatinine Mean±SD (mg/dL) | Weight Mean± SD (gms) | CKMB Mean±SD (mg/dL) |
|-----------------------|-------------------------------------|--------------------------|-------------------------|
|-----------------------|-------------------------------------|--------------------------|-------------------------|

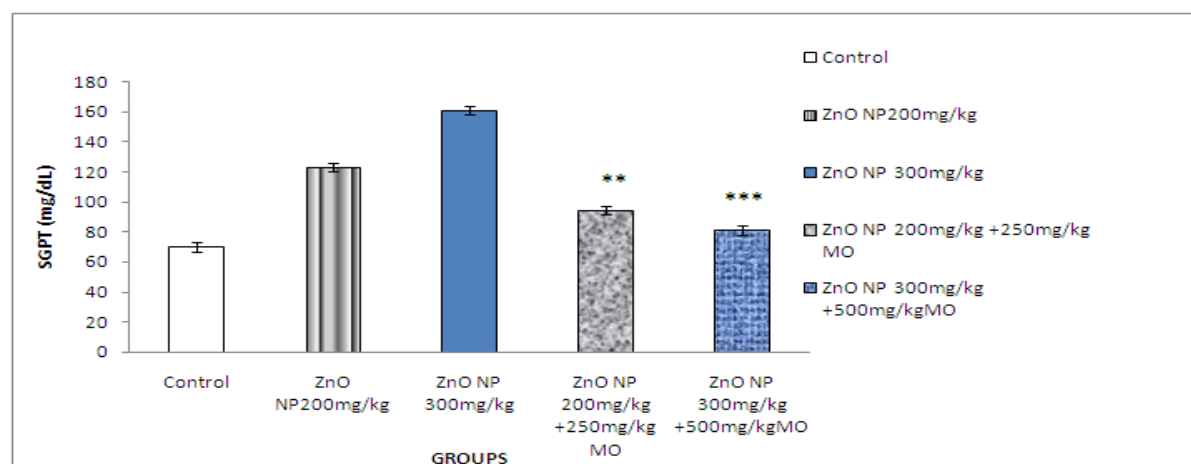
| | | | |
|---|---------------|------------------|----------------|
| | | | |
| Control | 0.62 ± 0.06 | 200.3 ± 5.3 | 225.3± 81.5 |
| ZnO NP 200mg/kg | 0.85 ± 0.08* | 181.3 ± 1.6* | 451.4± 72* |
| ZnO NP 400mg/kg | 1.32 ± 0.41** | 141 ± 2.3** | 622.6± 86.3** |
| ZnO NP 200mg/kg +250mg/kg NG | 0.91 ± 0.07** | 133.5 ± 3.2*** | 243.8± 33.2** |
| ZnO NP 400mg/kg +500mg/kgNG | 0.95±0.12** | 128.12 ± 2.2 *** | 453.4 ± 88.5 * |

Data were expressed in Mean ± SD (n=6),* P< 0.05, ** P< 0.01, *** P< 0.001 significant levels.



(Data were expressed Mean ± SD (n=6),* P< 0.05, ** P< 0.01, *** P< 0.001 significant levels)

Fig 1: Effect of *Moringa oliefera* on ZnO NPs induced Serum SGOT levels in swiss albino mice:



(Data were expressed Mean ± SD (n=6),* P< 0.05, ** P< 0.01, *** P< 0.001 significant levels)

Fig 2: Effect of *Moringa oliefera* on ZnO NPs induced Serum SGPT levels (Mean±SD)in swiss albino mice:

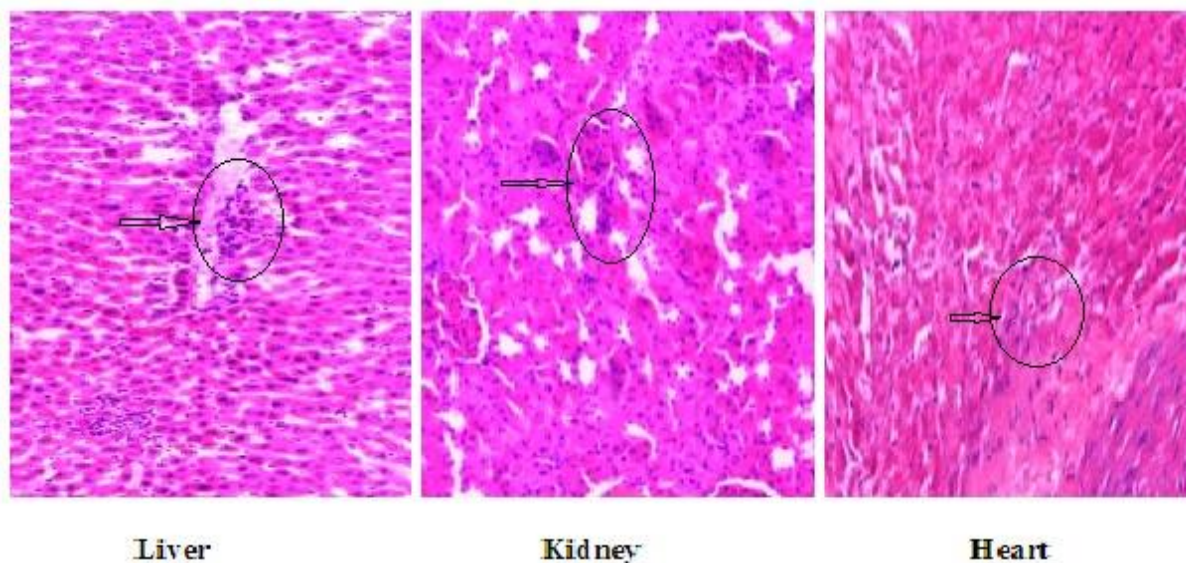


Fig3: Histology of Effect of *Moringa oleifera* on ZnO NPs induced toxicities in liver, Kidney and Heart of swiss albino mice:

DISCUSSION

Zinc itself is an important dietary supplement and zinc deficiency is a large public health concern in the developing world, but zinc oxide nanoparticles have been shown to be toxic to mammalian cells grown in the laboratory, because nanoparticles as a result of their extreme microscopic dimension, which gives unique advantage, have potential hazards similar to particulate matter. These particles have the potential to cause varied pathologies of respiratory, cardiovascular and gastrointestinal system. Sharma et al (2012) said that the DNA damaging potential of ZnO NPs is concluded by orally exposed mice for 14 consecutive days. In our study we were estimated different biochemical and histopathological changes which were supported by Vyom Sharma et al 2011 study revealed that, the nanoparticles when ingested into the body can be distributed to different regions because of their small size. They can cross the small intestine and further distribute into the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach. The toxicity mechanism of ZnO NPs may be mainly dependent on the interaction between NPs and biomolecules, and toxicity mainly involves protein unfolding (Chatterjee et al., 2010), fibrillation, thiol cross-linking, and enzymatic activity loss. Pre treatment with *Moringa oleifera* leaf extract in cadmium exposed rats act against kidney injury and has a positive effect on anaemia. *Moringa oleifera*, the most widely distributed species especially in Asian countries, is known to have a wide range of pharmacological properties with significant nutritional values and hence have been scientifically evaluated for various medicinal applications (Vinodini et al., 2014; Moussa Ndong et al., 2007). It not only has a positive effect in lowering the lipid levels but also alters the levels of the liver enzymes and hence can also improve the liver functions (Kuester et al., 2002; Oberdorster et al., 2005). In our study we found that the elevated levels of serum creatinine represented that 14 days exposure of ZnO nanoparticles orally in 150 mg/kg and 300 mg/kg groups have some renal

toxicity which was supported by the previous literature sharma 2012 , so .we use Moringaoliefera seed extract as preventive therapy for toxicity ,which contains phenolic compounds ,tannins, and glucosinates acts as antioxidants by quenching ROS . the leaf extract significantly decreased markers of gentamicin-induced kidney toxicity including histological changes, lipid peroxidation, and serum urea and creatinine levels. The feeding of an iron-deficient diet to rats results in hepatic ultrastructural changes (Ndong et al., 2007b).

The altered levels of SGOT and SGPT i.e increased levels of this paramaters found that toxicity of liver and it was confirmed by histopathology .It also shows that there is a histological changes because ofZno NPs nanoparticles . Due to this reason we are taking moringaoliefera seed extract as a preventive therapy because it contain antioxidant effect which was supported by the literature *Moringa oleifera* shows a decrease in the plasma levels of AST and ALT activities in substantial amounts in cadmium induced liver damage (Kuester et al 2002). In our study the elevated CK-MB levels indicated that there was a cardio toxicity and this was supported by histopathology shown that Oberdorster et al., 1995there was a nectriotic regions and inflammatory cells. Serum creatine phosphokinase isoenzyme (CK-MB) activity were measured kinetically to measure the cardiac toxicity. These results were supported to the A hydroalcohol extract of M. oleifera leaves has been shown to exhibit cardioprotective, antioxidant, and ntiperoxidative activity in response to isoproterenol in rats (Nandave et al., 2009). . M. oleifera leaf was shown to protect against chromium-induced testicular toxicity in rats (Sadek, 2013). (Jaiswal et al. 2013) have investigated the antioxidant activity of an aqueous extract of M. oleifera leaves in normal and diabetic rats. Oxidative free radical scavenging enzymes were measured in response to 200mg/kg of lyophilized powder. A significant increase in activities of superoxide dismutase, catalase, and glutathione S-transferase and a decrease in lipid peroxidation were observed. In our study the ZnO NPs induced toxicities were significantly reduced by usingMoringaoliefera seed extract, which was supported by biochemical and histiopathological studies.

CONCLUSION

In our study ZnO nanoparticles 200 mg/kg and 400 mg/kg showed toxicity, in preventive therapy *Moringa oleifera* seed extract 750 mg/kg showed protective activity. In our study the results support the ZnO nanoparticle toxicity was reduced by *Moringa oleifera* seed extract. Finally it was concluded that phenolic compounds in *Moringa oleifera* seed reduced the nanoparticle induced toxicities.

Conflicts of Interest: There is no conflict of interest.

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