The impact of *Moringa Oleifera* extract and vitamin E against zinc oxide nanoparticles induced hepatotoxicity in male albino rats

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Abstract: The present study aims to Evaluate the possible ameliorative effect of moringa extract and vitamin E alone or their combination against zinc oxide nanoparticles- induced liver injury in male Albino rats. 90 Male Wistar albino rats were divided into nine groups (n = 10). The 1st (normal control group) received distilled water. The 2nd (control group) in which rats received twin 80 orally using metallic stomach tube. The 3rd (moringa extract treated group) rats were treated orally with aqueous suspension of moringa extract in a dose of (150mg\kg. b.wt) daily for successive (30 days) using metallic stomach tube, the 4th (vitamin E treated group), rats were treated orally with vitamin E at a dose of (100mg\kg. b.wt). The 5th (ZnO Nps treated group), rats were orally administered with aqueous suspension of ZnO Nps in a dose of (7.5mg\kg. b.wt) daily for successive 15 day. The 6th ((ZnO Nps plus moringa extract treated group), Rats were orally administered with aqueous suspension ZnO Nps in a dose of (7.5mg\kg. b.wt) daily for successive 15 days then administered with aqueous moringa extract in a does of (150mg\kg. b.wt) daily for successive 30 days using metallic stomach tube. The 7th (ZnO Nps + vitamin E treated group), Rats were orally administered with aqueous suspension ZnO Nps in a dose of (7.5mg\kg. b.wt) daily for successive 15 days then administered with vitamin E in adoes of (100mg\kg. b.wt) daily for successive 30 days. The 8th (ZnO Nps + moringa extract & vitamin E treated group), Rats were orally Administered with ZnO Nps for 15 days then treated with aqueous suspension of moringa extract and vitamin E daily for successive 30 days and the 9th (ZnO Nps + Silymarin treated group) Rats were orally administered for ZnO Nps in adose of (7.5mg\kg. b.wt) for 15 days then treated with silymarin for 30 days. After 45 days, blood and specimens were collected. Lipid profile picture, ALT, AST, ALP, GGT, Total Protein, Albumin total bilirubin and direct bilirubin and some antioxidants assay (SOD, CAT, GST and GSH) were investigated In addition MDA. The results showed that administration of ZnO Nps caused an undesirable effect on most of studied biochemical parameters. The moringa and vitamin E administration for 30 days subsequent to ZnO Nps exposure afforded significant ameliorative effects on nearly all studied parameters and such effect were found compatible with the effect caused by silymarin as hepatoprotective drug.

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Abbreviations: Mo, moringa; NPs, nanoparticles; Vit. E, vitamin E; ZnO, zinc oxide; AST, aspartate transaminase; ALT, alanine transaminase; MDA, Malondialdhyde enzyme; SOD, Superoxide dismutase; CAT, Catalase; GSH, Glutathione reduced.

1. Introduction

There is growing community and scientific concern that the desirable technological characteristics of nanoparticles (NPs) may be offset by increased health and environmental risks, with the potential for exposure resulting in reactions at a cellular level that have not been observed with macroscopic materials ***(Natasha et al., 2007).*** The small size of NPs infers both greater mobility as well as potentially enhanced uptake across biological membranes ***(Chithrani et al., 2006).*** Zinc oxide (ZnO) NPs have a wide variety of applications in industry, including agriculture, medicine, and cosmetics. With increased use of ZnO NPs, exposure to these NPs has been rising steadily, resulting in more attention being paid to their potential toxicity, including cytotoxic, genotoxic, and proinflammatory effects ***(Hackenberg et al., 2011).***

Furthermore, ZnO is generally considered to be a material with low toxicity, because zinc (Zn) is an essential trace element in the human body and is commonly present in foods or added as a nutritional supplement, so Zn attracts little attention during assessment of toxicity of NPs ***(Baek et al., 2012)***. ZnO is slightly soluble and can release Zn^{2+} ions in solution. Some researchers considered that dissolved Zn ions in the toxicity of ZnO NPs played an important role. ***Brunner et al. (2006)*** inferred that

toxic effects of ZnO NPs on cells may be attributed to the dissolution of Zn^{2+} ions.

Moringa Oleifera Lam. is a drumstick tree of the Moringaceae family. It is a native of India and now grown extensively in many southeastern Asian countries particularly in Thailand, Philippines and Pakistan etc. Traditionally, *M.O.* is considered to be one of the most useful trees in the world, as almost every part of the tree has some nutritional, medicinal and other valuable properties. Additionally, the shade dried leaves of *M.O.* are widely utilized in developing countries as a good source of protein, calcium, vitamin A, C and E, β-carotene, amino acids, various polyphenolics and some natural anti-oxidizing agents. Therefore, it is used as an alternative source of nutritional supplements and growth promoters in various countries.

Apart from nutritional benefits, *M.O.* has been extensively used as an antioxidant, and for the following applications; wound healing, anti-tumor, anti-fertility, hypotensive, antipyretic, antihepatotoxic, antiepileptic, anti-inflammatory, antiulcer, diuretic, hypocholesterolaemic, antifungal, antibacterial and anti-cardiovascular agent, etc.. The extract of *M.O.* leaves is also capable of reducing hyperglycemia, dyslipidemia, and diabetes mellitus.

Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations ***(Kanter et al., 2005)***. One of the most important vitamins for the body is vitamin E. In nature, vitamin E comprises eight natural fat-soluble compounds, including 4 tocopherols [d-alpha-, d-beta-, d-gamma- and d-delta-tocopherol] and 4 tocotrienols [d-alpha-, d-beta-, d-gamma- and d-delta-tocotrienol] ***(Songthaveesin et al., 2004)***. Vitamin E is an important antioxidant factor. It is known to possess various physiological functions. A major contributor to non-enzymatic protection against lipid peroxidation is vitamin E, a known free radical scavenger ***(Rikans et al., 1991)***. Vitamin E as a lipid soluble, chain-breaking antioxidant ***(Packer, 1992)*** plays a major protective role against oxidative stress ***(Fraga et al., 1987)*** and prevents the production of lipid peroxides by scavenging free radicals in biological membranes ***(Suga et al., 1984)***. Since the discovery of vitamin E in 1922 by H.M. Evans, when it was first described as an anti-sterility agent, many scientists and physicians have sought to elucidate its biochemistry, health benefits and clinical applications ***(Packer, 1992)***.

There are multitudes of reports available on the protective effects of M.O. extract, vitamin E individually against various xenobiotics induced oxidative stress in experimental animals. Still to date the reports are scanty regarding the combined alleviated efficacy of M.O. extract in combination with vitamin E on ZnO NPs induced toxicity in rats. As well as there are some controversies over the combined administration of M.O. extract in combination with vitamin E. In view of the above considerations, the present study was designed to evaluate the protective efficacy of M.O. extract in combination with vitamin E on ZnO NPs induced toxicity and oxidative damages in the liver of rats.

2. Material and Methods

2.1. Drugs

2.1.1 Zinc oxide NPs:

Nanomaterials or nanostructures can be synthesized by a variety of techniques such as spray pyrolysis, thermal decomposition; molecular beam epitaxy, chemical vapor deposition, and laser ablation were purchased from sigma –Aldrich (St. louis MO, USA). The drug was given orally in a does level of (7.5mg/kg, b.wt) was selected on the basis of literature ***(Zhang et al., 2008).***

2.1.2- Vitamin E:

Vitamin E (dl-alph-tocopheryl acetate) is an essential nutritional element. Many of its actions are related to its antioxidant properties it helps thwart many common diseases by taming harmful molecules known as free radicals.

It was purchased from the local pharmacy. It is manufactured by PHARCO Company. Each 8 tablets were then dissolved in 100 ml twin80 then the drug was given orally in a dose level of (100mg/kg b.wt) was selected on the basis of literature ***(Ali, 2014).***

2.1.3 Silymarin preparation:

Silymarin was obtained from "Sedeco Pharmaceutical Co-6-october city. Egypt" silymarin was dissolved in distilled water and then administrated orally was selected on the basis of literature at dose (150 mg/kg) (each 1 ml contains 5.6 mg of silymarin) ***(Shimaa et al., 2013).***

2.1.4 Plant extract:

Moringae extract

M. O. Lam. (Moringaceae) is a rich source of essential minerals and antioxidants; it has been used in human and animal nutrition. The leaves and flowers are being used by the population with great dietary importance. The plant was collected from the pharmacognosy experimental farm. Pharmacy College Zagazig University in March 2013 (5kg.) was extracted by maceration in room temperature -3times each times 24hours by 70% ethanol. The total extract was concentrated under reduce pressure. The total extracts (250g). (36g) from extract dissolved in 900 ml distilled water the extract was given orally in a dose level of (150mg/kg b. wt.) was selected on the basis of literature ***Gupta et al., (2012)***.

2.2- Experimental animals:

The present study was carried out at Zoology Department, Faculty of Science - Zagazig University, using (90) ninety clinically healthy mature adult male albino rats (*Rattus norvegicus*).The animals were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University, Their weights ranged from 200-250g each.

The animals were housed in standard conditions, where the animals were housed in metal cages and bedded with wood shavings and kept under standard laboratory conditions of aeration and room temperature at about 25°C. The animals were allowed to free access of standard diet and water *ad libitum*.

The animals were accommodated to the laboratory conditions for two weeks before being experimented.

3- Experimental Design:

Experimental:

The study was performed on 90 mature male rats (*Rattus norvegicus*), divided into 9 main groups; each group was consisted of 10 rats.

2.1 - Treatment schedule:

I) The 1st normal Control group: served as control group in which rats received distilled water for (45days).

II) The 2nd control group: served as control group in which rats received twin 80 orally daily for successive (45days) using metallic stomach tube.

III) The 3rd *M.O.* extract treated group:Rats treated orally with aqueous suspension of *M.O.* extract in a dose of (150mg\kg. bw) daily for successive (30 days) using metallic stomach tube.

IV) The 4th vitamin E treated group: Rats were treated orally with vitamin E at a dose of (100mg\kg. b.wt) daily for successive (30 days) using metallic stomach tube.

V) The 5th ZnO Nps treated group: Rats were orally administered with aqueous suspension ZnO NPs in a dose of (7.5mg\kg. b.wt) daily for successive 15using metallic stomach tube.

VI) The 6th ZnO Nps plus *M.O.* extract treated group: Rats were orally administered with aqueous suspension ZnO NPs in a dose of (7.5mg\kg. b.wt) daily for successive 15 days then administered with aqueous *M.O.* extract in a dose of (150mg\kg. b.wt) daily for successive 30 days using metallic stomach tube.

VII) The 7th ZnO Nps plus vitamin E treated group: Rats were orally administered with aqueous suspension ZnO NPs in a dose of (7.5mg\kg. b.wt) daily for successive 15 days then administered with vitamin E in a does of (100mg\kg. b.wt) daily for successive 30 days using metallic stomach tube.

VIII) The 8th ZnO Nps plus *M.O.* extract & vitamin E treated group:Rats were orally administered with ZnO NPs for 15 days then treated with aqueous suspension of M.O. extract and vitamin E daily for successive 30 days.

XI) The 9th ZnO Nps plus silymarin treated group: Rats were orally administered for zno NPs in a dose of (7.5mg\kg. b.wt) for 15 days then treated with silymarin for 30 days.

Blood sampling:

Blood samples were collected after the end of the experiment (45 successive days)from the retro-orbital vein, which is a simple, convenient and successful procedure that allows bleeding of the same animal more than one time with minimal stress ***(Scherners, 1967).***

After the last administration of the drug at the end of the experiment, individual blood samples were drawn by orbital puncture (from eye plexus) using microhematocrit capillary tubes (Lancer, Athy, County-Kildare, Republic of Ireland), Serum was harvested from blood without EDTA and then serum samples were transferred into Eppendorf tubes and subsequently used for the determination of (Albumin, albumin, urea, uric acid,creatinine,Total protein, Aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphotase(ALP), (GGT), Total Protein, Albumin total bilirubin and direct bilirubin and Catalase, Superoxide dismutase, Malondialdhyde, reduced glutathione and glutathione –s-tranceferase.

Preparation of Tissue Homogenate:

Tissue parts of liver were used for the analysis of oxidative stress parameters. They were washed with saline and distal water for the removal of blood, and later the fatty parts were removed and blotted over a piece of filter paper. Prior to dissection, tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4 / NaH_2PO_4) (pH 7.4) in an Ice containing medium containing 0.16 mg / ml heparin or containing 0.1 mM ethylene di amine tetra acetic acid (EDTA) to remove any red blood cells and clots. Then tissues were homogenized in 5 – 10 ml cold buffer per gram tissue and Centrifuged at 5000 r.p.m for ½ hours. The resulting supernatant was transferred into Eppendorf tubes, and preserved at (-80) C in a deep freezer until used for various biochemical Assays ***(Habig et al., 1974).***

4-Methods

4.1.1 Determination of serum Amin otransferase Enzymes activities:

Activities of AST and ALT in the serum were determined colorimetrically by using bio Merieux kit (France), using method adopted by ***Reitman and Frankel (1957).***

4.1.2 Determination of alkaline phosphatase activity (ALP):

Alkaline phosphatase was determined in serum according to enzymatic colorimetric method according to ***Burstein et al. (1970)***, using spectrum kit.

4.1.3 Determination of serum gamma glutamyltransferase (GGT):

The gamma glutamyltransferase activity was determined spectrum kit, according kinetic colorimetric method described by ***Szasz et al. (1974).***

4.1.4 Determination of serum total protein concentration:

Serum total proteins were determined by Biuret method, using the Diamond kit ***Henry (1974).*** According to this method, protein forms a colored complex with cupric ions in an alkaline medium.

4.1.5 Determination of serum albumin:

Albumin estimation depends on the dye binding, as an essential method and according to ***Doumas and Watson (1971).***

4.1.6 Determination of serum total and direct bilirubin:

Serum total and direct bilirubin were determined by using the Spectrum kit, according to enzymatic colorimetric method according to ***Malloy and Evelyn (1937)***.

4.2. Markers of Lipid peroxidation and (Antioxidant Assay):

4.2.1 Determination of Lipid peroxide (Malondialdhyde) activity:

Malondialdehyde (MDA) was determined by using Biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of ***Satoh (1978)*** and ***Ohkawa et al. (1979).***

4.2.3 Determination of Catalase activity:

Catalase (CAT) activity was determined by biodiagnostic kit method (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of ***Aebi (1984).***

4.2.4 Determination of reduced glutathione (GSH).

Reduced glutathione was determined in tissue supernatant by colorimetric method using biodiagnostic kit, according to ***Beutler et al., (1963).***

3.4.4 Determination of glutathione –s- transferase:

Liver glutathione peroxidase (GST) wasdetdrmined using biodiagnostic kit, according to the method described by ***Habig et al., (1974)***

4.2.5 Determination of Superoxide dismutase activity:

Superoxide dismutase (SOD) activity was determined by biodiagnostic kit (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of ***Nishikimi et al. (1972).***

5 - Statistical analysis:

Data were collected, arranged and reported as mean ± standard error of mean (S.E.M) of nine groups (Each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS/ version 15.0) The statistical method was one way analyzes of variance ANOVA test (F-test), and if significant differences between means were found, Duncan's multiple range test (Whose significant level was defined as ($P<0.05$) was used according to ***Snedecor and Cochran (1982)*** to estimate the effect of different treated groups.

3.Results

Effect of ZnO nanoparticlrs (7.5mg/kg), moringa (150mg/kg), Vitamin E (100mg/kg) and their combinations on Liver functions

(a) Effect of ZnO NPs, moringa, Vitamin E and their combinations on serum ALT:

Serum alanine transferases (ALT) levels were markedly elevated after 45 day post NPs administration to normal rats when compared with control groups. Meanwhile, significant decrease in serum ALT level as compared with ZnO NPs treated group was recorded in normal rats in response to administration of sylimarin in addition to vitamin E and M.O. their combinations only while, they afforded a significant increase as compared with normal control group but this effect was less intense than that produced by ZnO NPs.

Whereas ZnO NPs plus *M.O.* treated group afforded the best safer treatment comparing with ZnO nanopaerticles group table (1) fig. (1).

(b) Effect of ZnO NPs, moringa, Vitamin E and their combinations on AST:

Table (1) and Fig (1) showed that ZnO nano particles treated group afforded highly significant increase in AST level as compared with normal control group. Meanwhile, ZnO NPs plus vitamin E, ZnO NPs plus vitamin E and *M.O.* and ZnO NPs plus sylimarin treated groups elucidate significant decrease in AST level as compared with ZnO NPs treated group only while they also, afforded significant increase in AST level as compared with normal control group, but the effect was much less intense as compared with ZnO NPs treated group.

At the same time, ZnO NPs plus *M.O.* treated group elucidate significant decrease in AST level as compared with normal control group and other groups and this is the best safer treated group.

(c) Effect of ZnO NPs, moringa, Vitamin E and their combinations on serum ALP:

Serum alanine transferases (ALP) levels were markedly elevated after 45 day post NPs administration to normal rats when compared with control groups. Meanwhile, significant decrease in serum ALP level as compared with ZnO NPs treated group was recorded in normal rats in response to administration of sylimarin in addition to vitamin E and M.O. their combinations only while, they afforded a significant increase as compared with normal control

group but this effect was less intense than that produced by ZnO NPs.

Whereas ZnO NPs plus *M.O.* treated group afforded the best safer treatment comparing with ZnO nanopaerticles group table (1) fig. (1).

(d) Effect of ZnO NPs, moringa, Vitamin E and their combinations on GGT:

ZnO NPs treated group elucidate significant increase in GGT level as compared with normal control group, Meanwhile, ZnO NPs plus vitamin E, ZnO NPs plus *M.O.* and ZnO NPs plus *M.O.* and vitamin E and ZnO NPs plus sylimarin treated groups afforded decrease in GGT level as compared with ZnO NPs treated group only, meanwhile, they induced increase in GGT level as compared with normal control group, but ZnO NPs plus *M.O.* treated group afforded the best safer treatment as shown in table (1) and Fig (2).

Table (1): Effect of ZnO NPs (7.5mg/kg), M.O.(150mg/kg), Vitamin E (100mg/kg) and their combinations on (combinations on Liver function parameters.

Groups	ALT	AST	ALP	GGT
Control	57.0±13.7^{e}	53.8±12.22^{c}	66.6±14.98^{d}	5.5800±1.09^{c,d}
Twin80	84.0±6.7^{d,e}	76.8±6.46^{c}	79.8±9.68^{c,d}	7.6200±.972^{a,b}
Vit E	81.4 ±8.6^{d,e}	67.0±7.68^{c}	79.6±5.46^{c,d}	5.6200±1.038^{c,d}
Moringa	73.0±7.0^{d,e}	63.6±7.06^{c}	78.2±6.85^{c,d}	4.5400±.618^{d}
ZnO NPs	177.8±14.17^{a}	163.8±10.41^{a}	131.8±4.58^{a}	8.3800±.292^{a}
ZnO NPs +moringa	101.6±5.07^{c,d}	81.0±9.76^{c}	93.0±6.14^{b,c}	6.1400±.606^{b,c}
ZnO NPs +vit. E	149.8±8.97^{a,b}	132.4±5.01^{b}	109.2±8.21^{a,b}	7.2800±.918^{a,b}
ZnO NPs +moringa+vit.E	130.2±7.19^{b,c}	109.2±6.20^{b}	108.6±4.03^{a,b}	6.2600±.637^{a,b,c}
ZnO+sylimarin	123.6±14.4^{b,c}	112.2±10.10^{b}	110.8±5.22^{a,b}	6.4800±.688^{a,b,c}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

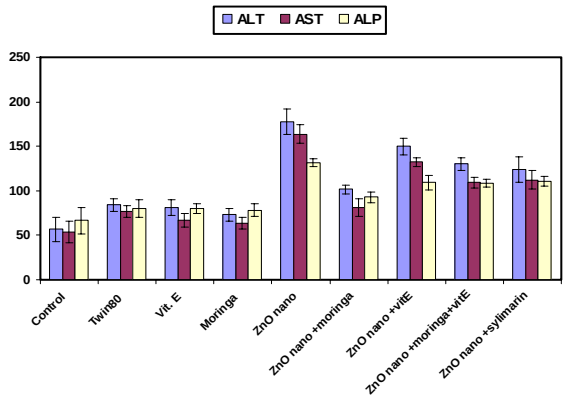


Fig (1)

(e) Effect of ZnO NPs, moringa, Vitamin E and their combinations on total protein:

ZnO NPs treated group and other treated groupes afforded a nonsignificant change in total protein level as compared with the control group as shown in table (2) and Fig (3).

(f) Effect of ZnO nanooparticles, moring, Vitamin e and their combinations on Albumin:

ZnO NPs treated group and other treated groupes afforded a nonsignificant change in albumin level as compared with r control group as shown in table (2) and Fig (4).

(g) Effect of ZnO NPs, moringa, Vitamin E and their combinations on total bilirubin:

ZnO nanoparticles treated group afforded significant increase in serum total bilirbin level as compared with normal control group, Meanwhile, ZnO NPs plus *M.O.* and vitamineE, ZnO NPs plus vitamin E, ZnO NPs plus sylimarin and ZnO NPs plus M.O. treated groups elucidate significant decrease in total Bilirubin level as compared with ZnO NPs which at the same time afforded a insignificant change in total bilirubin level as compared with normal control group as shown in table (2) and Fig (5).

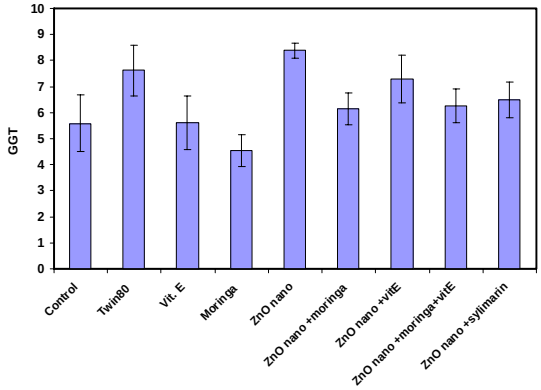


Fig (2)

(h) Effect of ZnO NPs, moringa, Vitamin E and their combinations on direct bilirubin:

ZnO nsanoparticles treated group afforded significant increase in serum direct bilirubin level as compared with normal control group, Meanwhile, ZnO NPs plus *M.O.* and vitamine E, ZnO NPs plus vitamin E and ZnO NPs plus *M.O.* treated groups elucidated significant decrease in direct bilirubin level as compared with ZnO NPs which at the same time afforded a insignificant change in direct bilirubin level as compared with normal control group as shown in table (2) and Fig (6).

Table (2): Effect of ZnO nanoparticlrs (7.5mg/kg), M.O. (150mg/kg), Vitamin E (100mg/kg) and their combinations on (combinations on Liver function parameters)

groups	Total protein	Albumin	Total bilirubin	Direct bilirubin
Control	7.58±.830[a]	4.84±.644[a]	1.38±.146[b]	0.460±.163[c]
twin80	6.94±1.04[a]	4.62±.620[a]	1.24±.215[b]	0.64±.172[b,c]
vitE	7.38±.412[a]	4.54±.708[a]	1.66±.174[b]	0.540±.112[b,c]
Moringa	6.62±1.13[a]	5.16±.382[a]	1.66±.261[b]	0.54±.136[b,c]
ZnO NPs	8.58±.611[a]	4.70±.518[a]	2.96±.608[a]	2.02±.769[a]
ZnO NPs +moringa	6.20±.641[a]	4.88±.751[a]	1.46±.188[b]	0.64±.107[b,c]
ZnO NPs +vit. E	6.94±1.33[a]	5.32±1.07[a]	1.18±.324[b]	0.66±.092[b,c]
ZnO NPs +moringa+vit.E	7.62±1.00[a]	5.66±.728[a]	1.12±.149[b]	0.60±.100[b,c]
ZnO+sylimarin	8.02±1.11[a]	4.90±.687[a]	1.96±.602[b]	1.60±.535[a,b]

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

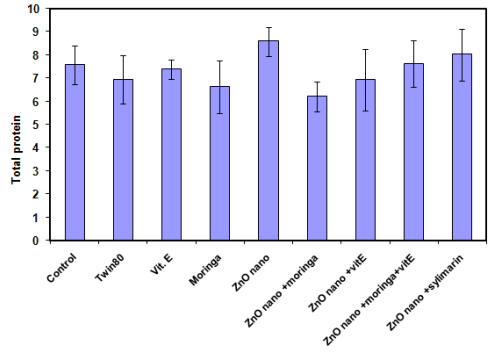
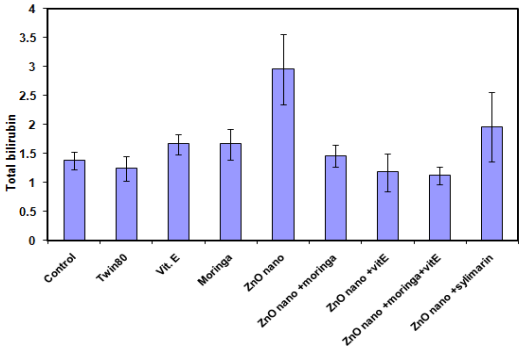


Fig (3)



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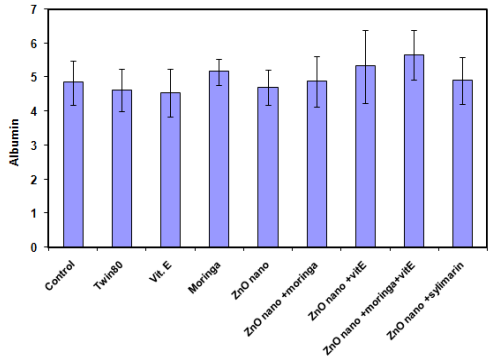

Fig (4)

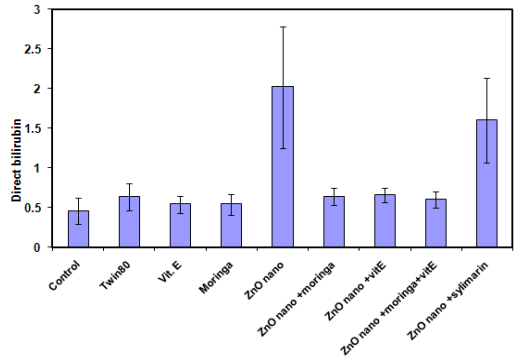

Fig (6)

Effect of ZnO nanoparticlrs (7.5mg/kg), moringa (150mg/kg), Vitamin E (100mg/kg) and their combinations on (combinations on Antioxidant parameters

(a) Effect of ZnO NPs, moringa, Vitamin E and their combinations on Malondialdhyde (MDA) activity:

The MDA content of the liver was significantly elevated ($P<0.05$) in response to treatment of normal male rats with ZnO nanoparticles compared with control groups. Meanwhile, ZnO nanoparticles plus moringa and ZnO nanoparticles plus vitamin E treated groups elucidated significant decrease in MDA level as compared with ZnO nanoparticles treated group only while they also, afforded significant increase in MDA level as compared with normal control group, but the effect was much less intense as compared with ZnO nanoparticles treated group.

At the same time, ZnO nanoparticles plus moringa and Vitamin E treated group elucidate non significant change in MDA level as compared with normal control –groups as shown in fig(3) and table(7).

(b) Effect of ZnO NPs, moringa, Vitamin E and their combinations on Catalase (CAT) activity:

ZnO nanoparticles treated group afforded highly significant decrease in CAT activity level as compared with normal control group. Meanwhile, ZnO nanoparticles plus vit E and ZnO nanoparticles plus sylimarin treated groups elucidate increase in CAT activity level as compared with ZnO nanoparticles treated group only while they also afforded decrease in CAT activity level as compared with normal control group, but the effect was much less intense as compared with ZnO nanoparticles treated group.

At the same time, ZnO nano particles plus moringa and Vitamin E treated group and ZnO nanoparticles plus moringa treated groups elucidate significant increase in CAT activity level as compared with normal control groups and ZnO nano particles plus moringa treated group showed the best safer treatment as shown in table (4) and fig (8).

(c) Effect of ZnO NPs, moringa, Vitamin E and their combinations on Glutathione reduced (GSH) activity:

ZnO nanoparticles treated group afforded highly significant decrease in GSH activity level as compared with normal control group. Meanwhile, ZnO nanoparticles plus vitE and ZnO nanoparticles plus sylimarin treated groups elucidate significant increase in GSH activity level as compared with ZnO nanoparticles treated group only while they also afforded significant decrease in GSH level as compared with normal control group, but the effect was much less intense as compared with ZnO nanoparticles treated group.

At the same time, ZnO nano particles plus moringa and Vitamin E treated group and ZnO nanoparticles plus moringa treated groups elucidate significant increase in GSH activity level as compared with normal control groups and ZnO nano particles plus moringa treated group showed the best safer treatment as shown in table (4) and fig (9).

(d) Effect of ZnO NPs, moringa, Vitamin E and their combinations on Glutathione S transferase (GST) activity:

ZnO nanoparticles treated group afforded highly significant decrease in GST activity level as compared with normal control group. Meanwhile, ZnO nanoparticles plus vitE and ZnO nanoparticles plus sylimarin treated groups elucidate significant increase in GST activity level as compared with ZnO nanoparticles treated group only while they also afforded significant decrease GST activity as compared with normal control group, but the effect was much less intense as compared with ZnO nanoparticles treated group.

Table (3): Effect of ZnO nanoparticlrs (7.5mg/kg), M.O. (150mg/kg), Vitamin E (100mg/kg) and their combinations on MDA.

Groups	MDA
Control	10.39 ± 0.27^{d}
Twin80	15.49 ± 1.12^{c}
VitE	14.90 ± 1.97^{c}
Moringa	12.60 ± 0.18^{cd}
ZnO NPs	31.24 ± 0.84^{a}
ZnO NPs +moringa	22.80 ± 0.92^{b}
ZnO NPs +vit. E	23.45 ± 1.52^{b}
ZnO NPs +moringa+vit.E	20.34 ± 3.04^{c}
ZnO+sylimarin	$27.61 \pm .62^{ab}$

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

At the same time, ZnO nano particles plus moringa and Vitamin E treated group and ZnO nanoparticles plus moringa treated groups elucidate significant increase in GST activity level as compared with normal control groups and ZnO nano particles plus moringa treated group showed the best safer treatment as shown in table (4) and fig (10).

Table (4): Effect of ZnO NPs (7.5mg/kg), M.O. (150mg/kg), Vitamin E (100mg/kg) and their combinations on antioxidant activity.

Groups	SOD	CAT	GST	GSH
Control	495.20±32.7[a]	10.78±1.31[a,b]	14.40±1.14[b]	10.90±.434[a]
Twin80	456.60±23.0[a]	10.32±.704[a,b]	11.66±.915[b,c]	9.54±.406[a,b]
VitE	473.60±9.57[a]	10.48±.673[a,b]	13.32±1.10[b,c]	10.72±.576[a]
Moringa	476.00±10.2[a]	12.16±.560[a]	17.66±1.55[a]	10.74±.227[a]
ZnO NPs	247.60±19.6[C]	6.74±.949[d]	6.32±.792[d]	3.44±.362[d]
ZnO NPs +moringa	453.60±7.80[a]	9.98±.912[a,b,c]	13.20±1.57[b,c]	9.48±.443[a,b]
ZnO NPs +vit. E	362.40±19.0[b]	7.32±1.06[c,d]	10.14±.488[c]	7.20±.525[c]
ZnO NPs +moringa+vit.E	439.20±8.52[a]	9.62±.813[a,b,c]	11.28±.500[b,c]	8.58±.859[c,d]
ZnO+sylimarin	368.60±25.8[b]	9.30±.356[b,c,d]	10.56±.722[c]	7.56±.615[c]

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

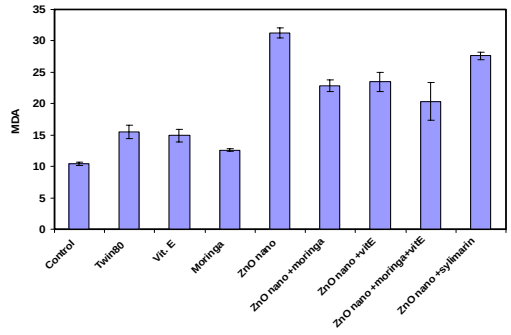

Fig (7)

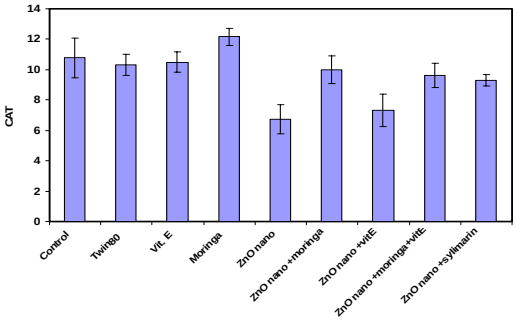

Fig (8)

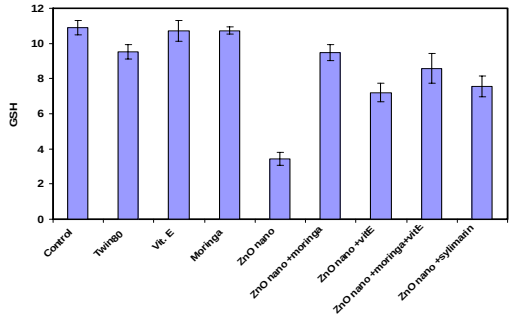

Fig (9)

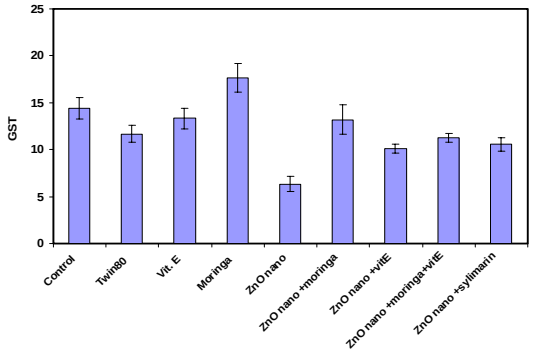

Fig (10)

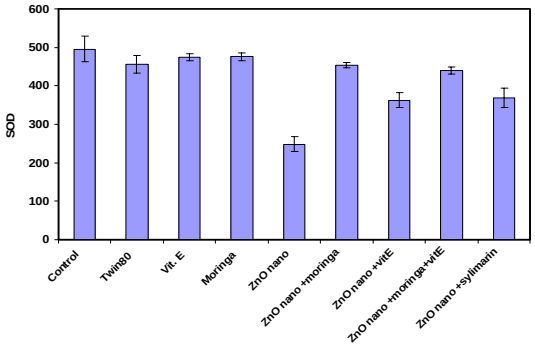

Fig (11)

(d) Effect of ZnO NPs, moringa, Vitamin E and their combinations on Superoxide dismutase (SOD) activity:

Table (4) and Fig (11) showed that ZnO nanoparticles treated group afforded highly significant decrease in SOD activity level as compared with normal control group. Meanwhile, ZnO nanoparticles plus vitE and ZnO nanoparticles plus sylimarin treated groups elucidate significant increase in SOD activity level as compared with ZnO nanoparticles treated group only while they also afforded significant

decrease in SOD activity as compared with normal control group, but the effect was much less intense as compared with ZnO nanoparticles treated group.

At the same time, ZnO nano particles plus moringa and Vitamin E treated group and ZnO nanoparticles plus moringa treated groups elucidate significant increase in SOD activity level as compared with normal control groups and ZnO nano particles plus moringa treated group showed the best safer treatment.

4. Discussion

The present study was an attempt to evaluate toxicity of ZnO nanoparticels on some physiological and biochemical parameters and the possible ameliorative role of *Moringa olifera* extract and vitamin E in alleviating the toxicity of ZnO nanoparticels when given to normal rats. Their effect on the following parameters, some liver functions parameters (ALT, AST, ALP, GGT, total proteins and albumin), some kidney functions parameters (serum urea, creatinine, uric acid and blood urea nitrogen) and lipid profile (Triglycerides, total cholesterol and total lipid) as well as some antioxidant parameters (SOD, MDA, GSH, CAT and GST) were also studied.

After the end of the experiment (15 successive days for ZnO nano treated group and 45 successive days for other groups) blood samples were collected from each rat. The blood samples were harvested without anticoagulant for collecting (serum) used for estimating various biochemical parameters.

All rats were sacrificed and tissue specimens were collected from liver for preparing tissues homogenates for measuring antioxidant enzymes.

NPs are important scientific tools that have been recruited in various biotechnological, pharmacological applications. They have two particular properties including their large surface area that dominates the contributions made by the small bulk of the material and their quantum effects ***(Erb et al., 2002)***.

The toxicological effects of zinc oxide NPs (ZnO-NPs) are attracting increasing concern as the field of nanotechnology progresses. Although the literature suggests that toxicity of ZnO-NPs may be related to their dissolution, the mechanism for ZnO-NP perturbation of cytosolic zinc concentration ([Zn2+]c) homeostasis remains obscure. There was a great toxicity of ZnO NPs by in-vitro studies on different biological system like bacteria and mammalian cells ***(Sinha et al., 2011)***.

At present, no specific therapy is available especially for acute liver failure without any adverse effects. Therefore, the need is urgent for effective therapeutic agents from natural products for the treatment of liver diseases. Natural drugs are frequently considered to be less toxic and freer from side effects than synthetic drugs. Recently, the search for effective crude drugs of plant origin through nutritional aspects with strong antioxidant nature has become a central focus for the study of hepatoprotection ***(Kuriakose and Kurup, 2011)***.

M.O. leaves act as a good natural source of antioxidant vitamins/compounds such as ascorbic acid, flavonoids, phenolics and carotenoids. The higher concentration of ascorbic acid, estrogenic substances, and β-sitosterol, vitamins, and particular essential amino acids such as methionine, cysteine, tryptophan and lysine present in *M.O.* leaves and pods make it virtually an ideal nutritional supplement ***(Adejumo et al., 2012)***. Therapeutic use of *M.O.*edible parts in many regions of Africa as well as Asia, it is widely consumed for self-medication by patients affected by diabetes, hypertension, or HIV/AIDS ***(Kasolo et al., 2010)***. It possesses various biomedical properties such as anti-inflammatory, antioxidant, antimicrobial, antifertility, anticancer, antihepatotoxic, and antiulcer activities ***(Goyal et al., 2007).***

Vitamin E has received wide attention due to its reported hepatoprotective effects in animals, which is primarily due to its ability to attenuate the induced oxidative stress in various tissues by reducing MDA levels; restoring the levels of GSH, SOD, and CAT and the recovery of impaired hepatic cells ***(Bharrhan et al., 2010)***.

Many studies have reported the antioxidant protective effect of vitamin E against several metals that induced hepatotoxicity, including copper, lead, sodium fluoride, cadmium, mercury, and ferrous sulphate ***(Al-Attar, 2011)***. The protective effect of vitamin E against Cr-induced hepatotoxicity was reported in a study where the authors investigated the direct protective effect of vitamin E on Cr (VI)-induced cytotoxicity and lipid peroxidation in primary cultures of rat hepatocytes ***(Susa et al., 1996)***. ***Susa et al. (1996)*** reported that such protective effects may be associated with the levels of nonenzymatic instead of enzymatic antioxidants.

Effect on some liver functions:

The present data suggests that ZnO-NPs can cause liver function impairment via elevation of liver function biomarker levels including ALT and AST, ALP ang GGT. And these results seem to be conceivable with that obtained by ***Sharma et al. (2012)*** and ***Fazilati (2013).***

Serum aminotransferases (ALAT & ASAT) are cytosolic enzymes of hepatocytes; an increase in their activities reflecting an increase in the plasma membrane permeability of hepatocyte which in turn associated with cell death ***(Rosen & Keeffe, 2000)***.

Due to their small size, NPs can translocate from these entry portals into the circulatory and lymphatic systems, and ultimately to body tissues and organs

(Donaldson et al., 2005). Some NPs, depending on their composition and size, can produce irreversible damage to cells by oxidative stress or/and organelle injury ***(Powers et al., 2006)***. Here we investigated toxicity effect of Zinc oxide NPs on ALT, ALP and AST enzymes in male Rat. Understanding the specific mechanisms of NPs and its interaction Require very extensive research in this field. When the NPs are accumulated in a tissue, may be absorbed into the cells or not to be absorbed. If these particles are absorbed, the finally replacement in cell lysosomes or cell cytoplasm will depend on the characteristics of NPs. If the NPs are located in the cytoplasm, the presence of some coarse grain material can cause direct damage or cell death is caused by this interactions.

In this study, to evaluate the toxicity effect of nanomaterials on the rat's liver, the ALT, ALP and AST were measured. ALT and AST were located in cell and ALP was located in cell membrane. In effect the loss of liver cells, these enzymes are released in the blood. Therefore, increases of these enzymes are a sign of liver cells damage. ALT and AST indicate Status of liver cells. ALP further demonstrates the performance and biliary Hungarian injuries, especially Hungarian extra hepatic. We conclude that the development of nanotechnology and the study of nanotoxicology have increased our awareness of environmental particulate pollution generated from natural and anthropogenic sources, and hope that this new awareness will lead to significant reductions in human exposure to these potentially toxic materials.

The reversing of hepatotoxic effect induced by Zinc oxide NPs, herewith observed after treatment with moringa, sylimarin and vitamin E which evaluated by significant decreasing in liver marker ALT, AST, ALP, GGT and serum proteins comparing with Zinc oxide NPs only treated group. And these results seem to be conceivable with that obtained by ***Pari and Kumar (2002), Fakurazi et al. (2008)***, ***Selvakumar and Natarajan (2008), Jaiswal et al. (2009)*** and ***Sheikh et al. (2014).*** They reported that *M.O.*extract had a hepatoprotective effect liver injury.

In addition, *M.O.*flowers contain a well-recognized flavonoid (Quercetin), which may be responsible for its potent hepatoprotective activity ***(Selvakumar and Natarajan, 2008)***.

At the same time, the present results revealed that treatment with vitamin E alone or/and in combination with *M. O.* extract after ZnO-NPs administration was able to normalize the levels of liver enzymes biomarker. These demonstrated by significant decrease in ALT, AST, ALP, GGT and significant increase in total bilirubin, direct bilirubin as compared with ZnO-NPs treated group. And these results were supported with that reported by ***Susa et al. (1996)***, ***Omer et al. (2000)*** and ***Al-Attar (2011).*** They reported that vitamin E has hepatoprotective effect due to its antioxidant activity.

Many studies have reported the antioxidant protective effect of vitamin E against several metals that induced hepatotoxicity, including copper, lead, sodium fluoride, cadmium, mercury, and ferrous sulfate ***(Al-Attar, 2011)***.

From the present results we could conclude that, there is a synergistic effect between *M.O.*and vitamin E which appeared in the results obtained from their combination in the pretreatment in ZnO-NPs induced hepatotoxicity, which in turn afforded the best ameliorative effect.

The effect on lipid peroxidation and antioxidant status:

The present results suggested that ZnO-NPs significantly decreased the activities of SOD, catalase, GSH and GST and enhanced LPO in the hepatic tissues indicating ZnO-NPs-induced oxidative damage. In contrast, a marked increase in the antioxidant enzyme activities was seen when rats were treated with vitamin E and/or *M.O.* alone and their combination after ZnO-NPs administration.

In the same context, the inhibition of enzymatic antioxidant (CAT, and SOD) system and reduction of the non enzymatic antioxidant system such as GSH and GST here with, after ZnO-NPs administration in full agreement with that reported by ***Nel et al. (2006), Xia et al. (2008), Alarifi et al. (2013) and Lai et al (2015).*** They reported that ZnO NPs were found to induce oxidative stress, evidenced by generation of reactive oxygen species and depletion of the antioxidant, glutathione.

However, oxidative stress can occur as a result of either increased ROS generation and/or decreased antioxidant enzyme system comprising SOD, catalase and GSH. These antioxidant enzymes protect the cell against cytotoxic ROS. SOD and catalase together convert superoxide radicals first to H_2O_2 and then to molecular oxygen and water. Other enzymes such as GSH use thiol-reducing power of glutathione to reduce oxidized lipids and protein targets of ROS. Under ineffective antioxidant enzyme status, lipid peroxidation in the cellular and subcellular membranes is the inevitable outcome of ROS injury ***(Fadillioglu et al., 2004).***

Reduced glutathione (GSH) is considered as a sensitive oxidative stress marker because it helps to maintain the integrity of mitochondria and cell membrane. Its compromised level in the cells may deteriorate the membrane permeability and risks the cellular defense against ROS resulting in oxidative injuries ***(Younes and Siegers, 1981)***. Many glutathione based antioxidant enzymes and proteins are important to maintain redox status of the cells. All these enzymes utilize glutathione (GSH) in the

reactions they catalyze which may lead to depletion of GSH in the living system in the condition of oxidative stress. This may be the possible reason for decreased level of GSH in target organs in ZnO NPs treated animals.

Xia et al. (2008) reported that ZnO NPs induce generation of reactive oxygen species which can lead to cell death when the antioxidative capacity of the cell is exceeded. Glutathione, a ubiquitous and abundant antioxidant cellular tripeptide, was found to be strongly depleted after exposure to ZnO NPs. Superoxide dismutase is specialized to convert the highly toxic superoxide radical to less toxic H_2O_2. The catalase enzyme reduces H_2O_2 to H_2O. More production of intracellular reactive oxygen species and more membrane lipid peroxidation in cells exposed to ZnO NPs along with depletion of their antioxidant components suggest that oxidative stress might be a primary mechanism for the toxicity of ZnO NPs.

Another way by which reactive oxygen species are generated is through activation of nicotinamide adenine dinucleotide phosphate oxidase, which is the enzyme responsible for O2 production in the membrane of phagocytic cells. In the case of ZnO NPs, generation of reactive oxygen species has been attributed to their semiconductor and nanolevel characteristics, which lead to generation of reactive oxygen species even in the absence of light. Moreover, the phenomenon of dissolution is expected to become more prominent in the case of NPs because of its dependence on surface area ***(Alarifiet al., 2013).***

Regarding the effect of moringa, according to the present results treatment with M.O.after ZnO-NPs administration afforded a significant increase in antioxidant enzymes (SOD and CAT) GSH and GST level and significant decrease in lipid peroxidation (MDA) level comparing with ZnO-NPs only as slymarin. And these finding strongly supported by ***Pilaipark et al. (2008), Arti et al. (2009), Vinay et al. (2012), kumar et al. (2013) and Pakade et al. (2013).*** They demonstrated the in vitro and in vivo antioxidant properties of water extract of M.O.

Phenolic compounds are known for their potent antioxidant properties. This class of plant metabolites contains more than eight thousand known compounds, ranging from simple phenol to complex materials such as tannins. ***Balasundram et al. (2006)*** showed that phenolics are the main dietary antioxidants and possess higher in vitro antioxidant capacity than the essential vitamins and carotenoids. Various findings have also shown that the phenolic-rich hydroethanolic fractions of plants contain catechin, rutin, quercetin, kaempherol and isorhemnetin ***(Zu et al., 2006).*** In this present study, it is shown that *M.O.* extract hasa high total phenolic content and antioxidant capacity. The presence of the active phenolic compound quercetin, a powerful antioxidant, in *M.O.* extract was reported by ***Ruckmani et al. (1998).*** These findings were correlated with previous studies ***Verma et al. (2009)***. Mainly concerning phenolic compounds and their antioxidant properties. The *M.O* leaves contain different classes of phytocompounds, vitamins and carotenoids ***(Dillard and German, 2006)*** and these compounds mainly contribute to the antioxidant properties as well as other biological activities. β-Carotene from *M.O.* leaves is efficiently converted into vitamin A in the body and has shown significant hepatoprotective effects. These reported studies have strongly suggested the importance of phenolic compounds from *M.O.* and their antioxidant properties. The main antioxidant activity that has been associated with phenolic content ability is to scavenge free radical formation ***(Verma et al., 2009)***. Certain phenolic compounds also may induce production of glutathione-S-transferase and other antioxidant enzymes. The ability of certain phenolic compound to bind to minerals may be beneficial in some cases, since copper and iron can be initiators of hydroxyl radical production by the Fenton and Haber-Weiss reactions ***(Ferguson, 2001).***

At the same time, this increase of the antioxidant enzymes SOD, catalase, GST and GSH activities and decrease of lipid peroxides with administration of vitamin Ein ZnO-NPs induced oxidative stress in the present study was in agreement with ***Xinchen et al. (2001), Kline et al. (2004), Peralta et al. (2006)*** and ***Bharrhan et al. (2010).*** They reported that vitamin E has antioxidant activity.

Vitamin E functions as a chain-breaking antioxidant that prevents the propagation of free radical reactions ***(Burton et al., 1983).*** In addition***, Kline et al. (2004)*** reported thatvitamin E; a free-radical scavenger in the lipid compartment of cells and serum is known for its beneficial antioxidant effects for number of chronic diseases, including cancer.

Conclusion

Therefore, the results of the present study demonstrated that the moringa extract in combination with vitamin E had a significant ameliorative action on ZnO NPs induced oxidative damage and toxicity in rats. ZnO NPs increased AST, ALT, ALP, GGT, in addition decreased albumin, total protein, as well as decreased the GSH, GST, CAT and SOD levels also lipid peroxidation product (MDA) increased. Association of antioxidant/anti-radical properties of moringa extract and vitamin E. Further the hepatic and renal protection was maximum in the combined treatment of moringa extract in combination with vitamin E than the moringa extract or vitamin E alone in the ZnO NPs intoxicated rats.

Recommendations

We recommend the use of the combination of moringa extract and vitamin E which are known as antioxidants compounds in amelioration of ZnO NPs toxicity.

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