ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Zinc Oxide Nanoparticles-Induced Neurotoxicity And Possible Mitigating Effects Of Artemisia judaica And Vitamin C.

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ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) have been extensively applied to many industrial domains such as alloys, ceramics, paints, rubber as well as biological fields including medicine, personal care products, sunscreens, and food additives. They can pass through the cell membrane easily and even pass through blood – brain barrier. They are highly reactive and may cause oxidative stress which induce serious damages in DNA and protein structures forming mutation. So, the current study was designed to assess the possible mitigating effect of *Artemisia judaica* (Art.j.), vit.C or their co-administration against ZnO NPs –induced neurotoxicity in rats. Eighty adult male rats were divided into 8 groups : the 1st (control group); the 2nd (twin 80 group) ; the 3rd (ZnO NPs group); the 4th (vit. C group); the 5th (*Art. j.* group); the 6th (ZnO NPs + vit. C group); the 7th (ZnO NPs + *Art. j.* group) ; the 8th (ZnO NPs + vit. C- *Art. j.* co-administration group). ZnO NPs group showed high significant decrease in the levels of serotonine, dopamine, epinephrine, nor-epinephrine, high density lipoprotein, very low density lipoprotein, insulin and testosterone. While showed high significant increase in triglycerides, total cholesterol, low density lipoprotein and blood glucose levels as compared with control group. Vit. C and Art.j. groups did not show any significant changes in the previous parameters except slight changes in some lipid profile indices and blood glucose levels. ZnO NPs group that were treated by vit.C or Art.j. showed partial ameliorative effect against ZnO NPs – induced neurotoxicity with convergent degree. ZnO NPs group that were treated by vit. C- Art. j. co-administration showed markedly significant curative effects against ZnO NPs-induced neurotoxicity.

Keywords: Nanoparticles, zinc oxide nanoparticles, neurotoxicity, *Artemisia judaica,* vitamin C.

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INTRODUCTION

Nanoparticles are very important scientific materials that have been used in various fields such as biotechnology and pharmacological applications. They have specific characters as their large surface area that predominates the contributions made by material small size and their influences ***(Erb et al., 2002 and Saman et al., 2013)***. These agants affect the chemical reactions of materials and their electrical, magnetic, mechanical and optical properties. In nanoparticles, the fraction of the atoms at the surface is enhanced compared to their bulk. Nanoparticles have a wide surface area and elevation of particles number/unit mass when compared with microparticles. Elevation of surface area to volum rate of the particle size reduction happens gradually predominated by atoms behaviour in the particle surface to the activity of inner atoms. Whereas, chemical reactivity usually enhances with lessening particle volum, surface coatings and other modulations can have complicating impacts, even decreasing reactivity with reducing particle volume in some cases ***(Erb et al., 2002 and Alivisatos, 1996).***

Dioxide nanoparticles have unrivaled properties that can make wide variance of cosmetic, colors, ceramics and many other industrial employments ***(Mital and Manoj, 2011)***. For example, Zinc oxide nanoparticles (ZnO NPs) have been found to have outstanding UV blocking characters compared with its bulk material. For this reason it is usually used in the synthesis of sunscreen lotions ***(Chang et al., 2012 and El Shemy et al., 2017)***. Despite these widespread uses of ZnO NPs in industries, many hypothesis have been elevated about the possible of their danger and toxic effects on environments and their living organisms, so, it is very important to realize the toxicity of these nanomaterials in order to development of safe and sustainable nanotechnology ***(Chen at al., 2006 and Zhang et al., 2010)***.

Though, there are some studies and information about toxicological impact of nanomaterials are predictable to interact with the materials of biological components to produce significant impacts on the properties and behaviour of macromolecules, cells, tissues, organs and body ***(Revell 2006 and Saman et al., 2013)***.

ZnO NPs is one of the most widespread used nanomaterials in many industries domains eg. pigments, paints, dyes, textile, metallurgy additives, alloys, rubber, chemical fibers, ceramics, catalyst, electronics, sunscreens, beauty agants, medical diagnosis, cosmetics, food additives, toothpaste and personal care products ***(Djurisic and Leung 2006 and Fan and Lu 2005)***.

The widespread applications of ZnO NPs might elevate the possibility of human exposure through halation, skin contact and ingestion. Although ZnO NPs do not penetrate skin layers, but small fractions of sunscreen lotion and lip products may be accidentally ingested. Moreover, ZnO NPs can be easily absorbed with polluted food or water. The liver, kidney, spleen and brain are the main target organs for these nanomaterials after absorption through the cells of gastrointestinal canal ***(Filipe et al., 2010, Emamifar et al., 2011 and Kairyte et al., 2013, Tian et al., 2015, Tang et al., 2016 and Santhoshkumar et al., 2017)***.

Administration of ZnO NPs to wistar rats through oral gavage cause accumulation of these nanoparticles in hepatic tissue leading to oxidative stress and deterioration of liver cells ***(Guan et al.,2012, Ben-Slama et al., 2015 and El Shemy et al., 2017)***.

ZnO NPs that were added to the feed of rats caused oxidative stress to liver tissues appeared in deleterious effects on histological parameters of liver and kidney tissues, hematological parameters, liver enzymes activity, cytochrome enzymes and cytokines levels ***(Jang et al., 2014 and Tang et al., 2016)***.

Other studies reported that ZnO NPs induce cytotoxic impacts in many kinds of cells, as bronchial epithelial cells of human, osteoblast cancer cells, hepatorenal cells and embryonic cells of kidney, all these effects may be related to the dosages and the size of these nanoparticles ***(Nair et al., 2009, Heng et al., 2011, Guan et al., 2012, Kang et al., 2013, Al-Rasheed et al., 2014, Al-Rasheed et al., 2016 and El Shemy et al., 2017)***.

The toxicity of ZnO NPs has been widely studied in different animal organs and tissues. The studies revealed that ZnO NPs could reach to different organs after systemic distribution and may be showed deletcrious effects on liver, lungs, kidney, pancrease, stomach, spleen, thymus, testis, blood, heart and brain

(Cho, 2011, Vandebriel and De Jong 2012, Li , 2012 ,Wang, 2008 and Ansar et al., 2016). Moreover, studies have been proved that nanoparticles can reach to the brain tissue through blood brain barrier permeation or translication via the pathway of olfactory nerve and subsequently induce deteriorate effect due to induction of oxidative stress, cytotoxicity and inflammatory responses ***(Hu and Gao 2010 and Costa et al., 2014)***. It has also been reported that these nanoparticles could reach to the brain tissues through inhalatory or oral administration in rats ***(Lee et al., 2012 and Kao, 2012)***, induce changes in the ability of memory and spatial learning of rats by damaging the synaptic plasticity ***(Han et al., 2011)***, and diversely interact with plasma and proteins of brain during stimulating their toxic effects in the brain and blood ***(Shim et al., 2014)***. Moreover, inflammation reaction and oxidative stress were found in mice brain after ZnO NPs exposure and many noticeable changes were showed in aged individual. These types of brain toxicity might be due to devastation of the blood-brain barrier integrity caused by the systemic inflammation induced by ZnO NPs. Additionally, ZnO NPs exposition caused neuronal pathological changes and abnormal cognitive function in the brain hippocampus ***(Win-Shwe and Fujimaki, 2011 and Tian et al., 2015)***.

Artemisia judaica, belongs to the family Asteraceae, and is a fragrant shrub that grows widely in the Arabian area, phytochemical analysis of *Art. J.* showed that it is a rich source of flavonoids including apigenin, cirsimaritin, and various novel compounds ***(Sujatha et al., 2007 and Grech and Pietrosiuk, 2012)***.

Members of the Asteraceae family produce sesquiterpene lactones and flavonoids are the most interesting ones from the pharmacological point of view ***(Torrell and Vallès 2001 and Baluchnejad-mojarad et al., 2005)***. These substances are known for their reported medical efficacy e.g. strong anti-inflammatory, antimalarial, antioxidant, antitumor activity, as well as for the fact that they increase immunity and decrease the risk of atherosclerosis, arthritis and gastrointestinal disorders ***(Stojanowska, 2010 and Kazemi et al., 2011)***. All *Artemisia* species produce aromatic oils, and several are culinary herbs or used as flavorings, hallucinogens, vermifuges, and pharmaceuticals; many species cause allergies to humans and some are toxic ***(Sanz et al., 2008 and Pellicer et al., 2011)***.

Vit C is a water soluble antioxidant agent that accumulates in the brains of mammals more than any other tissue ***(Siddigue et al., 2007; Abdou et al., 2009 and Grosso et al., 2013)***. Vit C is a co-factor of some enzymes such as dopamine-β-hydroxylase and collagen synthase which are essential for the life ***(Braakhuis, 2012)***.

Vitamin C is an essential dietary nutrient for the biosynthesis of collagen and a co-factor in the biosynthesis of catecholamines, L-carnitine, cholesterol, amino acids, and some peptide hormones, the deficiency of vitamin C causes scurvy, a pathological condition leading to blood vessel fragility and connective tissue damage due to failure in producing collagen, and, finally, to death as result of a general collapse ***(Grosso et al., 2013)***. Vit C serves in humans also as a co-factor in several important hydroxylation reactions, such as the biosynthesis of catecholamines (through the conversion of dopamine to norepinephrine), L-carnitine, cholesterol, amino acids, and some peptide hormones ***(Arzi et al., 2004; Harrison and May, 2009 and Shahidi et al., 2008).***

So this work was designed to assess the ameolirative effect of *Artemisia judaica* (Art.j.), vit.C or their co-administration against ZnO NPs –induced neurotoxicity in rats.

MATERIALS AND METHODS

Experimental animals:

Eighty adult male rats (*Rattus norvigicus*) weighting 200-250 g were used throughout the present study. They were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University, Egypt.

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.

Chemicals and plant extraction

Zinc oxide nanoparticles

Zinc oxide nanoparticles were purchased from Sigma –Aldrich (St.louis MO, USA). The drug was given orally in a dose level of (10 mg/kg) ***(Zhang et al., 2008)***.

Vitamin C

Effervescent tablets single vitamin (vitacid c) was purchased from the local pharmacy. It is manufactured by CID Company. Each tablet contains 1g ascorbic acid. Each tablets was then dissolved in 50 ml distilled water then the drug was given orally in a dose level of (100 mg/kg b. wt.) ***(Rana and Ahmad., 2012)***. The dose administration was as follow: (20 mg of vitamin C was equivalent to 1 ml of prepared solution).

***Artemisia judaica* extract**

The plant material of *Artemisia judaica* collected from its natural habitat (Saint Catherine, South Sinai) during spring period by Pro.Dr.Samih Ibrahim El-Dahmy Professor of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt.

Fresh cut aerial parts (leaves and stems) were extracted by maceration at room temperature -3 times each times 24 hours by ethyl alcohol 96%. For the botanical study, the total extract was concentrated under reduced pressure, fresh samples were used. The total extract was 100g. 1.5g from extract was dissolved in 50 ml destill water the extract was given orally at a dose level of 150 mg / kg b. wt.

(Abd-Alla et al., 2014) The dose administration was as follow: (30 mg of Art.j. was equivalent to 1 ml of prepared solution).

Experimental design:

Animals were divided into 8 main groups (10 rats each) as follows:

1) **The 1st (control group):** rats were gavaged daily with distilled water for 45 days.
2) **The 2nd (twin 80 group):** rats were gavaged daily with twin 80 for 45 days.
3) **The 3rd (ZnO NPs group):** rats were gavaged daily with 10 mg/kg b.wt of ZnO NPs for 15 days.
4) **The 4th (vit. C group):** rats were gavaged daily with 100 mg/kg. b.wt of vit.C for 30 days.
5) **The 5th (*Art. j.* group):** rats of were gavaged daily with 150 mg/kg. b.wt of *Art. j.* extract for 30 days.
6) **The 6th (ZnO NPs + vit. C group):** rats were gavaged daily with 10 mg/kg. b.wt of ZnO NPs for 15 days then gavaged daily with 100 mg/kg. b.wt of vit C for 30 days.
7) **The 7th (ZnO NPs + *Art. j.* group):** rats were gavaged daily with 10 mg/kg. b.wt of ZnO NPs for 15 days then gavaged daily with 150 mg/kg. b.wt of *Art. j.* for 30 days.
8) **The 8th (ZnO NPs + *Art. j.*- vit. C co-adminstration group)*:*** rats were gavaged daily with 10 mg/kg. b.wt of ZnO NPs for 15 days then gavaged daily with *Art. j.* and vit C co-administration for 30 days.

Blood sampling:

Blood samples were collected at the end of the experiment 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).***

Serum was harvested from blood without EDTA and then serum samples were transferred into eppendorf tubes and subsequently used for the determination of triglycerides, total cholesterol, low denisty lipoprotein (LDL), high denisty lipoprotein (HDL), very low denisty lipoprotein (VLDL-c), insulin, blood glucose & testosterone.

ISSN: 0975-8585

Preparation of tissue homogenate:

Tissue parts of brain were immediately weighed to avoid drying and were homogenized in ice-cold solution of acidified n-butanol to obtain 10% homogenate. Homogenization was performed using a glass homogenizer fitted with a glass pestle. Duplicate internal standard of serotonin, norepinephrine and dopamine was prepared by adding 0.3 ml standard mixture (0.1ml containing 100µg of each amine) to 9.7 ml of 0.2N acetic acid. Aliquots of 0.2 ml of this solution were diluted to 0.3 ml with 0.2N acetic acid then received 3 ml of acidified n-butanol. Acidified n-butanol was prepared by adding a volume of 0.85 ml of concentrated hydrochloric acid to one liter of n-butanol and cooling during mixing **.** This result solution was used for estimation of neurotransmitters ***(Habig et al., 1974)***.

Biochemical analysis

- ***Estimation of brain neurotransmitters:***
  -Estimation of dopamine, serotonin, epinephrine and norepinephrine contents according to ***(Ciarlone, 1978)***.

- ***Lipid Profile parameters:***
  -Determination of serum triglycerides concentration:
  Serum triglycerides were determined using Biocon kit, according to ***(Fossati and Prencipe, 1990).***

  -Determination of serum total cholesterol concentration:
  Serum cholesterol was determined using Bicon Kit. According to ***(Varley and Richmond, 1976)***.

  -Determination of HDL, LDL and VLDL:
  Serum HDL-cholesterol, LDL, and VLDL were determined according to ***(Stein, 1986)***

- ***Determination of glucose levels:***
  Serum glucose level was determined using diamond kit, according to ***(Trinder, 1969)***.

  -Determination of insulin hormone:
  Insulin was assayed using insulin-I^{125} kit according to ***(Woodhead et al., 1974)***
- ***-Determination of testosterone hormone:***

The assay used for the determination of testosterone hormone, using available kit according to ***(Pierce and Parson, 1981)***.

Statistical analysis:

SPSS version 22 software was used for statistical analysis. Suitable statistical techniques were calculated as mean, ± SD. One way ANOVA & LSD as post hoc test were used as tests of significance. Pearson correlation was done to correlate the different laboratory parameters with each other ***(Snedecor and Cochran 1982)***.

RESULTS

Data presented in this study showed that ZnO NPs induced neurotoxicity in rats appeared in high significant ($P<0.05$) decrease in the levels of brain tissue serotonine, dopamine, epinephrine, nor-epinephrine activities and serum levels of HDL, VLDL-c, insulin & testosterone as compared with control group (table 1-3). Whearas, showed. high significant ($P<0.05$) increase in the levels of serum triglycerides, total cholesterol, LDL and blood glucose (table 1-3) as compared with control groups.

Group 4 and 5 that were gavaged only by vit.C & Art.j. respectively showed some minor changes in some previous parameters when compared with control group.

Rats that were gavaged with ZnO NPs then treated with vit.C or Art.j. (Group 6 & 7) showed partial ameliorative effects in most of previous parameters as compared with control and ZnO NPs groups. Whereas (Group -8) that were gavaged by ZnO NPs then treated with co-administration of vit.C & Art.j. manifested pronounced curative effects to ZnO NPs – induced neurotoxicity in rats appeared in amelioration of all previous parameters that were affected by ZnO NPs as compared with control and ZnO NPs groups.

Table (1): Effect of ZnO NPs, *Art. j.*, vit C or their co-adminstration on some neurotransmitters (serotonine, dopamine, epinephrine& nor-epine-phrine) in brain tissue of rats:

Parameters / Groups	Mean ± SD (n = 10)			
	Serotonin(5-HT) (µg/ g tissue)	Dopamine(DA) (µg/ g tissue)	Epinephrine (µg/ g tissue)	Nor Epinephrine (µg/g tissue)
Control	0.526±.006	1.505±.015	0.637±.006	1.18±.0215
Twin 80	0.478±.007 $^{a+}$	1.462±.011 $^{a-}$	0.595±.006 $^{a-}$	1.23±.005 $^{a-}$
ZnO NPs	0.240±.006 $^{a+}$	0.588±.027 $^{a+}$	0.430±.009 $^{a+}$	0.723±.008 $^{a+}$
Vit C	0.553±.106 $^{a-}$	1.571±.018 $^{a-}$	0.613±.003 $^{a-}$	1.08±.718 $^{a-}$
Art. j	0.515±.005 $^{a- b-}$	1.471±.012 $^{a- b-}$	0.605±.005 $^{a-b-}$	1.07±.016 $^{a- b-}$
ZnO NPs + Vit C	0.312±.003 $^{a+ c+}$	0.826±.021 $^{a+ c+}$	0.521±.004 $^{a+ c+}$	0.847±.024 $^{a+ c+}$
ZnO NPs + Art. j	0.397±.006 $^{a+ b+ c+}$	1.030±.338 $^{a+ b+ c+}$	0.583±.005 $^{a- b- c+}$	0.88±.012 $^{a+ b+ c+}$
ZnO NPs + Vit C + Art.	0.458±.003 $^{a+ b- c+}$	1.253±.012 $^{a+ b- c+}$	0.60±.005 $^{a- b- c+}$	0.96±.010 $^{a+ b+ c+}$

$^{a+}$: Significant compared with control value ($P < 0.05$) $^{a-}$: Non significant compared with control value ($P < 0.05$).
$^{b+}$: significant compared with twin value ($P < 0.05$). $^{b-}$: Non Significant compared with twin value ($P < 0.05$).
$^{c+}$: Significant compared with ZnO NPs value ($P < 0.05$).

Table (2): Effect of ZnO NPs, vit C, *Art. j.* or their co-administration on serum TG, total cholesterol, LDL, HDL and VLDLc in serum of rats.

Parameters / Groups	Mean ± SD (n = 10)				
	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	LDL (g/dl)	HDL (g/dl)	VLDLc (g/dl)
Control	88.00±2.6	64.50±1.3	14.75±.25	48.87±2.0	9.112±.02
Twin 80	91.25±.41 $^{a-}$	71.00±.32 $^{a+}$	11.37±.49 $^{a-}$	48.50±1.2 $^{a-}$	10.45±.01 $^{a+}$
ZnO NPs	145.0±1.8 $^{a+}$	133.0±.46 $^{a+}$	69.12±.61 $^{a+}$	25.00±.26 $^{a+}$	5.387±.03 $^{a+}$
Vit C	73.12±1.1 $^{a+}$	61.50±.37 $^{a-}$	16.75±.36 $^{a-}$	40.37±1.0 $^{a+}$	9.762±.01 $^{a+}$
Art. j	78.87±.29 $^{a- b+}$	58.75±.31 $^{a+ b+}$	16.87±.44 $^{a- b+}$	51.12±.58 $^{a- b-}$	10.10±.04 $^{a-b-}$
ZnO NPs +Vit C	91.75±.41 $^{a- c+}$	80.87±.39 $^{a+ c+}$	21.75±.52 $^{a+ c+}$	46.25±1.2 $^{a- c+}$	7.487±.04 $^{a+ c+}$
ZnO NPs +Art. j	99.87±1.4 $^{a+ b- c+}$	75.62±.49 $^{a+ b- c+}$	23.00±.37 $^{a+ b+ c+}$	46.25±1.2 $^{a- b- c+}$	8.225±.07 $^{a+ b+ c+}$
ZnO NPs + Vit C + Art. j	91.75±.41 $^{a- b- c+}$	73.50±.86 $^{a+ b- c+}$	16.94±.11 $^{a+ b+ c+}$	55.50±1.1 $^{a+ b+ c+}$	8.625±.02 $^{a- b+ c+}$

$^{a+}$: Significant compared with control value ($P < 0.05$). $^{a-}$: Non significant compared with control value ($P < 0.05$).
$^{b-}$: Significant compared with twin value ($P < 0.05$). $^{b+}$: Non significant compared with twin value ($P < 0.05$).
$^{c+}$: Significant compared with ZnO NPs value ($P < 0.05$).

ISSN: 0975-8585

Table (3): Effect of ZnO NPs, *Art. j.*, vit C or their co-adminstration on blood glucose, insulin and testosterone level of serum rats:

Parameters / Groups	Mean ± SD (n = 10)		
	Glucose (mg/dl)	Insulin (IU/ml)	Testosteron (µIU/ml)
Control	105.00±.70	44.50±.86	4.575±.088
Twin 80	96.37±.59 [a-]	46.25±.55 [a-]	5.012±.039 [a-]
ZnO NPs	149.50±.98 [a+]	23.00±.88 [a+]	2.587±.044 [a+]
Vit C	95.00±.46 [a-]	44.62±1.1 [a-]	4.553±.042 [a-]
Art. j	99.87±1.8 [a- b-]	46.25±.52 [a- b-]	4.456±.099 [a- b+]
ZnO NPs + Vit C	126.25±.55 [a+ c+]	36.25±.36 [a+ c+]	3.987±.071 [a+ c+]
ZnO NPs + Art. j	119.5±.86 [a+ b+ c+]	31.62±.49 [a+ b+ c+]	3.512±.030 [a+ b+ c+]
ZnO NPs + Vit C + Art. j	109.12±1.02 [a- b+ c+]	32.00±.70 [a+ b+ c+]	4.144±.054 [a- b- c+]

[a+] : Significant compared with control value ($P < 0.05$). [a-] : Non significant compared with control value ($P < 0.05$).

[b-] : Significant compared with twin value ($P < 0.05$). [b+] : Non significant compared with twin value ($P < 0.05$).

[c+] : Significant compared with ZnO NPs value ($P < 0.05$).

DISCUSSION

ZnO NPs used in this study have small particle size, large specific surface area, positive charge, homogeneous suspension and more easily uptaken by cells, so, it induces neurotoxic effect. NPs not only pass through the BBB ***(Osmond-Mcleod et al.,2014 and Lai et al., 2016)*** and placental barrier ***(Cui et al., 2014 and Karmakar et al., 2014)*** but are also transported along the nerve and deposited in the brain. The most well-known neural transport pathway is the olfactory nerve pathway ***(Kanazawa et al., 2013 and Kreyling, 2016)***. NPs can translocate in the CNS (hippocampus and striatum) through the olfactory nerve pathway after inhalation or intranasal instillation exposure. It has been well demonstrated that ZnO NPs that accumulate in the brain can cause pathological changes, cell damage and apoptosis and ultimately lead to neurodegeneration through reactive oxygen species production and the oxidative stress induction play a critical role in the common mechanism of NPs toxicity ***(Wang et al., 2014, Tian et al., 2015 and Aijie et al., 2017)***

The blood brain barrier is the interface between the brain and vascular system and it is definitive for regulating transport of substance to brain. Many evidences designated that NPs including ZnO NPs in the circulation may produce variation in the permeability and integrity of blood brain barrier through the following mechanisms: ZnO NPs may be induce endothelial oxidative stress ***(Ng, 2011)*** which cause inflammatory pathway ***(Giovannia, 2015 and Setyawati et al., 2015)*** and endothelial cell leakiness ***(Tay et al., 2014)***. In addition NPs and proinflammatory moderators could infiltrate to the brain tissues causing neurotoxicity, the significant deleterious changes of determined serum parameters as a result of ZnO NPs may be the main reason for brain barrier damage. So, ZnO NPs can induce CNS toxicity by direct enter through the brain tissue or/and indirect through systemic inflammation impact ***(Tian et al., 2015)***.

This study clarify the determinted effects of ZnO NPs on brain tissues that appeared as significant decrease in the levels of neurotransmitters (Serotonine, dopamine, epinephrine and nor-epinephrine) as well as serum insulin, testosterone, HDL and vLDL were significantly decreased. While, the serum levels of triglycerides, total cholesterol, LDL and blood glucose were significantly increased as a result of ZnO NPs treatment. These results could mainly attributed to induction inflammation and oxidative stress as a result of ZnO NPs treatment which cause genotoxicity in brain tissues leading to neurodegeneration progression. ZnO NPs exposure can excite free radical activity in the tissues that seep into them. Oxidative stress and free radicals that resulting from it may be induce deterioration in lipid profile, proteins and nucleic acids at the

position of particle deposition and at the places where they are moving. ***(Tian et al., 2015; Win-Shwe et al., 2011 and Urrutia et al., 2014)***. The brain is essentially vulnerable to oxidative stress and reactive oxygen species because its high energy request, high cellular contant content of protein and lipids , in ddition, low level of antioxidant agants ***(Karmakar et al., 2014 and Migliore et al., 2015)***.

Moreover, NPs can deteriorate dopaminergic neurons in CNS through considerably high levels of free radicals which produced by microglial activation ***(Block et al., 2004)***. Furthermore, Oxidative stress and reactive oxygen species have been experimentally engaged in the neurodegenerative damage ***(Win-Shwe and Fujimaki, 2011)*** nanoparticles have the potential to generate reactive oxygen species via a metabolic pathway and induce oxidative stress, including oxidative DNA damage.

These results also could mainly attributed to ZnO NPs that may induce inflammation, apoptosis and oxidative stress by releasing various mediators from microglia and astrocyte in the brain. Depends on production of toxic (e.g., NO, excitatory neurotranmsitters) or anti-toxic mediators (e.g., anti-inflammatory cytokines, neurotrophins), it may leads to neurodegeneration or neuroregeneration.

The current study revealed the harmful effects of ZnO NPs on lipid profile, blood glucose, insulin & testosterone. Where the data showed high significant increase in serum triglycerides, toal cholesterol, LDL, blood glucose and high significant decrease in serum HDL, Vldl-c, insulin and testosterone

The significant reduction of serum testosterone level after administration of ZnO NPs in the present study, may be explained by the direct effect of ZnO NPs mediated through oxidative stress on testicular tissues, as evidenced by increasing testicular MDA levels in rats treated with ZnO NPs. This in turn, led to overproduction of free radicals that interacted with the polyunsaturated fatty acids of Leydig cells membranes and suggested for testicular injury and the significant decrease in serum testosterone levels.

Another explanation was suggested by ***Ismail,(2012)*** and ***Alalwani,(2014)***, they reported that, the damage nerve cells of the hypothalamus is a pointer to the fact that it may alter the neuronal control of reproductive hormone secretion via the hypothalamic-pituitary-gonadal regulatory axis. Therefore, the destroyed neurons of the hypothalamus and such neuronal losses can resulted in disruption of the hypothalamic- pituitary-testis regulatory axis that controls the steroidogenesis of testicular Leydig cells. This will ultimately impact serum testosterone levels, because 95% of the androgen is produced by Leydig cells of the testes ***(Igwebuike et al., 2011 and Soliman et al., 2016)***.

Oxidative stress that were induced by ZnO NPs has been prescribed as the main mechanism responsible for metabolic disturbance. It could triggers the progression of abnormal lipid metabolism. The generation of reactive oxygen species would react with unsaturated fatty acid chain in membrane lipids causing lipid peroxidation, which promotes the disturbance of lipid profile, blood glucose and insulin levels ***(Nagi et al., 2017)***.

The results of this study exhibited the animals that were gavaged with *Artemisia judaica* and vitamin C after administration of ZnO NPs showed significant increase in serum testosterone levels in all groups as compared with ZnO NPs treated group. This may be explained by the antioxidant effects of vit.C and the phenolic compounds in *Artemisia judaica,* where they act as free radical scavengers ameliorating the oxidative damage of testicular tissues caused by ROS, thereby increasing serum testosterone levels.

The present results revealed that treatment with *Art. j.* alone or/and in combination with vit. C after ZnO NPs administration was able to reduce the levels of serum triglycerides, toal cholesterol & LDL, the level of blood glucose and increase the level of serum HDL, Vldl-c, insulin, testosterone moreover enhancement of brain tissue serotonine, dopamine, epinephrine & nor-epinephrine values that were damaged by ZnO NPs administration.

This defensive effect of *Artemisia j* is almost certainly due to the presence of Phenolic antioxidant and flavonoids compounds, which are competent of harmful free radicals in the body. Also polyphenolic compounds, especially flavonoids, originally have an inhibitory effect on cytochrome P450 system which eventually lead to the lessening of free radicals production. The antioxidant property of polyphenolic compound, are capable of neutralizing free radicals present in the cell environment and prevent their

damaging effects. These results seem to be conceivable with that obtained by ***Madani et al., (2006); Mohammed et al., (2009) and Abbas et al., (2017)*** they showed that the protective effects of *Art. j.* extract against neurotoxicity may be due to its ability to obstruct the bioactivation of thioacetamide, mostly by inhibiting the activity of Cyp450 and free radicals.

Flavonoids compounds can restore cells against glutathione diminution and rising the ability of antioxidant enzymes ***(Bahrami et al., 2011).*** Moreover, *Artemisia* possesses quercetin which inhibits lipid peroxidation by their antioxidant properties; it has a major responsibility against free radicals in various diseases ***(Romaiana et al., 2009 and Abbas et al., 2017)***.

Vit. C supplementation provided a significant reduction in both LDL cholesterol and triglycerides. This may be explained as vit. C acts as a regulator of catabolism of cholesterol to bile acid and has been demonstrated to be an important factor in lipid regulation ***(Reza et al.,2013 and 2014)***.

Vit C can in fact efflux from various types of cells, including neurons, because of its hydrophilic nature and negative charge at physiologic pH. Also, vit C appears to be allowed to enter into several brain cell lines, improving neurotransmission and leading to a number of effects on behaviors such as learning, memory and locomotion ***(Win-Shwe and Fujimaki, 2011)***.

Also, vit C serves in humans also as a co-factor in several important hydroxylation reactions, such as the biosynthesis of catecholamines (through the conversion of dopamine to norepinephrine), L-carnitine, cholesterol, amino acids, and some peptide hormones ***(McRae, 2007)***.

Vit. C has combined effects on the separate lipid profiles, For example, one of the protective mechanisms of HDL cholesterol is to cause inhibition of LDL oxidation**.** Therefore, the benefits of v. C supplementation on increasing HDL cholesterol concentrations can indirectly carry over to act as a benefit for LDL cholesterol reduction ***(Win-Shwe and Fujimaki, 2011)***.

Concerning the effect of vit. C and *Artemisia Judaica* as they reveres the effect of ZnO NPs by elevating serum insulin level. This may attributed to the synergistic effect between *Artemisia Judaica* and vit. C as they repair the hepatotoxic and renotoxic effect induced by ZnO NPs as previously mentioned, leading to normal blood glucose and insulin level ***(Reza et al.,2013 and 2014)***.

So it could be concluded that the anti-diabetic effect of single or multiple doses of aqueous and ethanolic extracts of *Artemisia judaica* were due to the flavonoids compounds. This results correlate with the finding that *Artemisia* active principle is effective for the treatment of diabetes and oral administration of volatile oil of *Artemisia* significantly decreased the blood glucose level in alloxanized rats ***(Ballester et al., 2004).***

Moreover, ***Nofal et al. (2009)*** showed that single oral administration of aqueous, ethanolic extracts, *(Artemisia Judaica)* significantly decreased the blood glucose level. Increased blood sugar in animals was induced by producing oxygen radicals in the body, which cause pancreatic injury through destruction of â-cells of the islets of Langerhans; leading to massive reduction in insulin release and increased blood sugar in animals***.***

CONCLUSION

Under the light of this research, it can be concluded that *Artemisia judaica* and vitamin C have partial curative impact without considerable side effects wheras, co-administration of *Artemisia judaica* with vitamin C give pronounced curative effects against neurotoxicity that were induced by ZnO NPs. However, more researches should be done about *Artemisia judaica* to determine the exact and principle mechanisms of it in this situation and provide that opportunity to develop new therapies from this plant for prevention and treatment of neurotoxicity.

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