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Comparative Effects of Origanum majorana Aqueous Extract and Insulin-Like Growth Factor-1 (IGF-1) on Neurotransmitters, Biochemical, and Enzymatic Parameters in Male Mice Exposed to Zinc Oxide Nanoparticles-Induced Stress.

Dr. Ashwaq Jabbar Almiahy[1], Ban Abdulhussein Salih[2], Zainab Abd Alameir Alabudi[3]

[1]Department of Physiology, Faculty of Veterinary Medicine, Shatrah University, Iraq,
Email: ashwaq.jabbar@shu.edu.iq
[2]Teaching Veterinary Hospital of Thiqar Province, Iraq, Email: banabd47@gmail.com
[3]Department of Microbiology, Faculty of Veterinary Medicine, Shatrah University, Iraq,
Email: zainababd@vet.shu.edu.iq

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ABSTRACT
Zinc oxide nanoparticles (ZnO NPs) have attracted tremendous attention due to their value use in cosmetics, medicine, food packaging among other industries. At the same time, the rising concern of their possible toxicity, especially their capacity to cause neurotoxicity in biological organisms, is worrisome. insulin-like growth Factor-1 (IGF-1) has been established as a neurotrophic factor that facilitates growth, differentiation, and survival of neurons. However its effectiveness in countering the neurotoxicity caused by zinc oxide nanoparticles (ZnO-NPs) is still unclear. Origanum majorana is claimed as a natural antioxidants herb, which has exhibited strong neoroprotective effects. The aim of this study is to investigate events to compare between Origanum majorana aqueous extract (OMAE) and IGF-1 on neurotransmitters, oxidative stress markers, and enzymatic changes in male mice subjected to stress by ZnO NPs. 40 male Swiss albino mice were randomly divided into four equal groups (n = 10/group): Control Group G1: Untreated, ZnO-NPs Group G2: (50 mg/kg/ intraperetonial) for seven days, ZnO-NPs + IGF-1 Group G3 (100 µg/kg, subcutaneous) for seven days post-ZnO-NP exposure, ZnO-NPs + OMAE Group G4: (200 mg/kg, orally) also for seven days post-ZnO-NP exposure. Results showed that treatment with OMAE and IGF-1 repaired levels of (dopamine, serotonin and norepinephrine), as well as ameliorated the oxidative stress biomarkers (GSH, MDA and TAC) and activities of (SOD, CAT and AChE) enzymes. However treatment with OMAE showed superior potency. In conclusion OMAE demonstrated preferable neuroprotective effects compared to IGF-1 in attenuating ZnO-NP-induced stress. These findings highlight the efficacy of OMAE as a natural therapeutic agent for nanoparticle-induced neurotoxicity.

Keywords: Zinc Oxide nanoparticles, neurotransmitters, IGF-1, Origanum majorana, mice.

INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) have attracted tremendous attention due to their value use in cosmetics, medicine, food packaging among other industries. These nanoparticles boast impressive physicochemical properties such as antimicrobial effects, electrical conductivity, and the ability to filter UV light (Sirelkhatim et al 2015). Nonetheless, the growing application of these nanoparticles is raising apprehensive questions on the possible toxicity effects on the user. The use of these particles is thought to induce oxidative stress which deeply affects biological systems (Sharma et al 2012). Some studies also show that ZnO-NPs should be crossed the blood-brain barrier causing oxidative stress, neurotransmitter disturbance, and enzymatic activity disruption (Zhao et al 2020; Kumari et al 2021). An unharnessed production of reactive oxygen species caused by and unbalanced antioxidant defense system gives rise to oxidative stress (Wang et al 2014). Dopamine, serotonin, and norepinephrine are neurotransmitters that have a great significance in mood, cognition, and behavior. Studies have discovered that exposure to ZnO NPs can alter these neurotransmitters, resulting in negative behavioral and cognitive outcomes (Han et al 2011). Other findings noted that these nanoparticles also alter biochemical markers such as glutathione (GSH), malondialdehyde (MDA,) and total antioxidant. In this context, the role of therapeutic plants particularly in phenolic glycosides such as flavonoids and phenolic acids

which exhibit antioxidant and free radical scavenging as well as metal chelating activities, has received attention in terms of potential chemopreventive agents (Khalaf et al., 2020). The phenolic components have confirmed protecting effects towards oxidative stress that are caused by many poisons by harvesting ROS and improving the hosts cell antioxidants (Subapriya et al., 2005). It is characterized that Insulin-like growth factor-1 (IGF-1), a neurotrophic factor that contributes to neuronal growth, differentiation and survival. Its therapeutic promise on reducing oxidative stress has been documented, but its efficacy against ZnO-NPs-induced neurotoxicity remains unclear (Pardo et al., 2023). Likewise, Origanum majorana is a common aromatic medicinal herb from mint family (Lamiaceae) (Gurib-Fakim, 2006). It is dispersed as an allele in Asia, Europe and North America and is commonly referred to as wild marjoram conduit wintersweet (Zowail et al., 2019). These herbs also show strong neuroprotective property due to natural antioxidants (El-Baz et al., 2022).

This study aims to compare the effects of Origanum majorana aqueous extract (OMAE), and IGF-1 on neurotransmitters, biochemical markers, and enzymatic parameters in male mice exposed to ZnO NP-induced stress. The findings will provide insights into the potential therapeutic applications of these interventions.

MATERIALS AND METHODS

Experimental animals and chemicals

Forty 12-weeks-old Swiss albino male mice (25 ± 2 gr) were housed in a temperature (22-25° C) and light-controlled (12 h: 12 h light/dark cycle) animal house with free access to food and water for one week prior to the experiment. The study was approved by Institutional Ethical Committee in the College of Veterinary Medicine, department of physiology of Shatrah University, Thiqar, Iraq, and all experimental protocols were in accordance with the Guidelines for the Care and Use of Laboratory Animals. The ZnO NPs (10–30 nm according to the specification sheet) were acquired from Skyspring Nanomaterials incorporation USA, as white to light yellow nano powder, with a purity of 99.8%, density of 5.606 g cm3 and spherical morphology. Scanning probe microscope (SPM) and atomic force microscope (AFM) were employed to characterize size average and surface morphology. Origanum majorana (dried plant) was purchased from a local market in Egypt. The samples were washed with distilled water, crushed mechanically and then suspended in distilled water until use (5 g/kg body weight). IGF-I was purchased from R&D Systems (Minneapolis, MN, USA)

Study Design

A total of 40 male mice were randomly divided into four groups (n = 10/group) as follows

G1: Control Group: Untreated.

G2: ZnO-NPs Group: Exposed to ZnO-NPs at (50 mg/kg, intraperitoneally) for seven days.

G3: ZnO-NPs + IGF-1 Group: Treated with IGF-1 at (100 µg/kg, subcutaneously) for seven days post-ZnO-NP exposure.

G4: ZnO-NPs + OMAE Group: Treated with OMAE at (200 mg/kg, orally) for seven days post-ZnO-NP exposure.

Blood sampling

At the end of the period of experimental treatment (4 weeks), the mice in each group were fasted overnight and then sacrificed via cervical decapitation to collect blood samples. Plasma samples were separated through centrifugation of the blood for 15 min at 3000 rpm using EDTA as an anti-coagulant. More blood samples were centrifuged at 3000 rpm for 15 min to get Serum samples. The plasma and serum samples were held at -80°C for subsequent biochemical analysis.

Neurotransmitter Assays : After the animals were sacrificed, brain tissue was extracted for the estimation of dopamine, serotonin, and norepinephrine contents by the HPLC method .

Oxidative Stress Markers: The level of glutathione (GSH) in serum was determined by the modified method of Sedlak (Sedlak and Lindsay1968), which is based on the use of Ellmans reagent-5,5 (dithio bis(2-Nitrobenzoic acid) DTNB), which reacts with glutathione to form a colored product whose absorbance is read at 412 nm. Malondialdehyde (MDA) levels estimated by the method used by Wang (Wang et al., 2009). Total antioxidant capacity (TAC) was measured by using different spectrophotometric antioxidant assays.

Enzymatic Activities: The method proposed by Sun et al. was followed for the determination of SOD activity (Sun et al., 1988). Assay of serum CAT levels was performed using the method of Wang (Wang et al., 2009). Acetylcholinesterase level was estimated by Ellman's method.

Statistical Analysis
Data analysis was done by one-way ANOVA, followed by the Tukey post hoc test, via SPSS 25.0. The results are expressed as mean ± SD. Significance was considered at ≤ 0.05.

RESULTS

Neurotransmitter Levels
Exposure to ZnO-NPs significantly reduced the levels of dopamine, serotonin, and norepinephrine in a statistically significant manner ($p \leq 0.05$). OMAE and IGF-1 ameliorate these levels, where OMAE showed higher efficacy (Table 1).

Table 1: Neurotransmitter Levels (ng/mg tissue)

Group	Dopamine	Serotonin	Norepinephrine
Control	80.5 ± 4.1 **a**	95.2 ± 3.5 **a**	72.6 ± 3.3 **a**
ZnO-NPs	47.8 ± 3.7 **d**	58.4 ± 4.2 **d**	42.1 ± 2.8 **d**
ZnO-NPs + IGF-1	70.2 ± 3.6 **c**	81.3 ± 4.1 **c**	64.5 ± 3.3 **c**
ZnO-NPs + OMAE	76.8 ± 3.5 **b**	90.1 ± 3.6 **b**	69.2 ± 3.1 **b**

Different letters refer to the significant differences at **P ≤ 0.05**.

Oxidative Stress Markers
Exposure to ZnO-NPs significantly elevated the MDA level and lowered GSH and TAC levels. This oxidative stress had remarkably been ameliorated by OMAE in comparison with IGF (Table 2).

Table 2: Oxidative Stress Markers

Group	GSH (µmol/mg)	MDA (nmol/mg)	TAC (mmol/L)
Control	9.8 ± 0.6 **a**	2.3 ± 0.8 **c**	1.85 ± 0.13 **a**
ZnO-NPs	4.2 ± 0.2 **d**	6.7 ± 0.5 **a**	0.84 ± 0.08 **c**
ZnO-NPs + IGF-1	8.1 ± 0.5 **bc**	3.4 ± 0.4 **b**	1.52 ± 0.07 **b**
ZnO-NPs + OMAE	9.2 ± 0.4 **b**	2.9 ± 0.4 **c**	1.74 ± 0.09 **ab**

Different letters refer to the significant differences at **P ≤ 0.05**.

Enzymatic Activities
ZnO-NPs exposure significantly inhibited SOD, CAT and AChE activities. Both IGF-1 and OMAE ameliorated enzymatic activities, with OMAE demonstrating preferable efficacy (Table 3).

Table 3: Enzymatic Activities (U/mg protein)

Group	SOD	CAT	AChE
Control	15.4 ± 0.5 a	7.2 ± 0.3 a	8.5 ± 0.4 a
ZnO-NPs	9.2 ± 0.4 d	3.8 ± 0.2 d	4.1 ± 0.3 d
ZnO-NPs + IGF-1	13.8 ± 0.5 c	6.2 ± 0.3 b	7.3 ± 0.4 b
ZnO-NPs + OMAE	14.6 ± 0.4 b	6.8 ± 0.3 b	7.9 ± 0.3 b

Different letters refer to the significant differences at **P ≤ 0.05**.

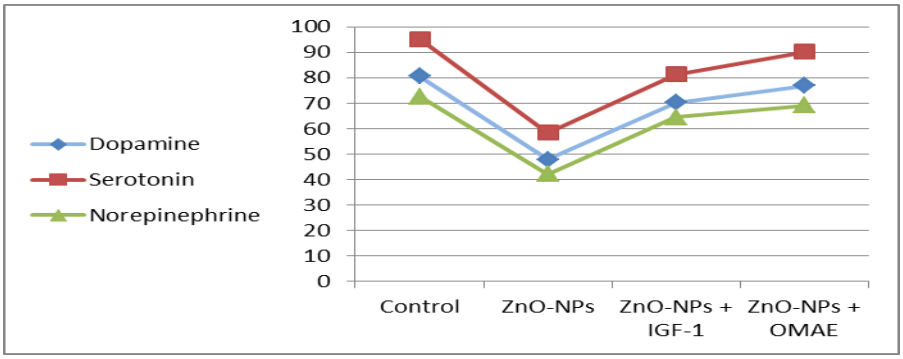

Figure 1: Neurotransmitter Levels (ng/mg tissue)

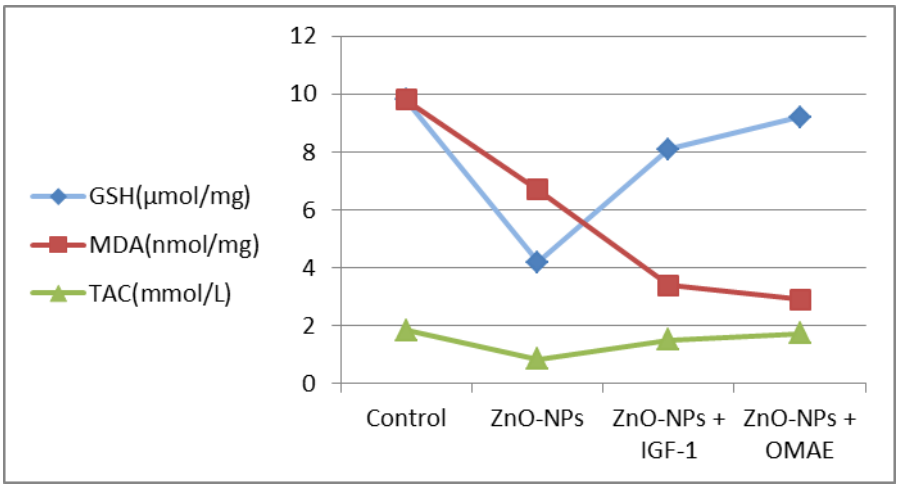

Figure 2: Oxidative Stress Markers

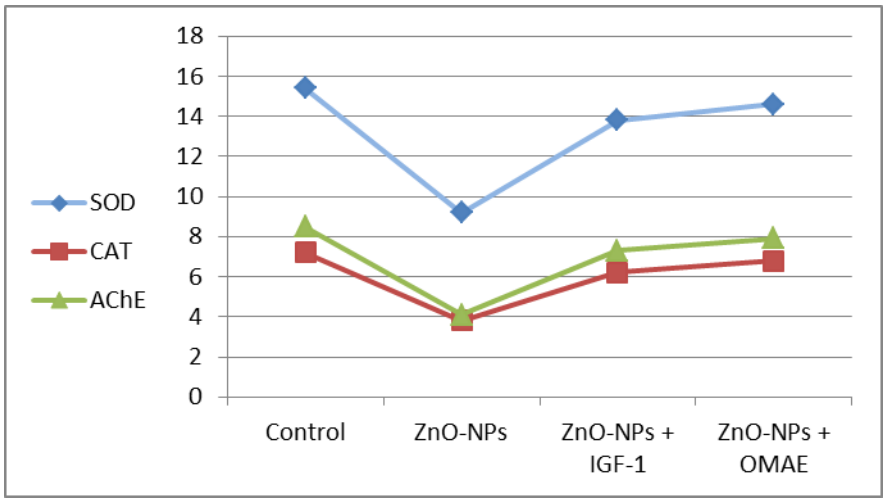

Figure 3: Enzymatic Activities (U/mg protein)

DISCUSSION

The significant findings of decrease of dopamine, serotonin, and norepinephrine levels in neural tissue after being exposed to ZnO-NPs were agreed with recent works that have revealed the neurotoxicity mechanism of ZnO-NPs via oxidative stress induction, mitochondrial damage, and disturbing neurotransmitter synthesis pathways(Kumar et al.,2022;Ameen et al., 2023). The most critical neurotransmitters, such as dopamine, serotonin, and norepinephrine, are involved in maintaining mood, cognition, and autonomic functions. It is very likely that the observed decrease of those neurotransmitters upon ZnO-NP exposure is related to the generation of ROS that impairs theactivityof the rate-limiting enzymes tyrosine hydroxylase and tryptophan hydroxylase in monoamine synthesis (Panza et al., 2023).

Levels of neurotransmitters in this search that restored by treatment with OMEA and IGF-1 are of interest, which was more effective when using OMAE than IGF-1(Table1).OMAE'ssuperior performance can be attributed to its rich content of bioactive compounds, including polyphenols, flavonoids, and terpenes, which exhibit potent antioxidant and anti-inflammatory properties (El Riachy et al., 2021). These compounds likely mitigate ROS-induced damage, protect mitochondrial function, and enhance the activity of neurotransmitter-synthesizing enzymes. In contrast, the neurotropic factor IGF-1 mainly exerts its effects on neuronal survival and synaptic plasticity through the activation of the PI3K/Akt signaling pathway (Lee et al., 2022). Although IGF-1 significantly repaired neurotransmitter levels, its single-target mechanism may explain its relatively lower efficacy compared to OMAE's multi-targeted approach. The differential restoration of neurotransmitter levels (OMAE > IGF1) underlines the therapeutic potential of natural extracts with multi-component actions in attenuating nanomaterial-induced neurotoxicity.

These results emphasize the need to investigate natural antioxidants as adjunctive or alternative therapies in Nan otoxicology. ZnO-NP exposure caused severe oxidative stress, as indicated by increased levels of malondialdehyde (MDA, a marker of lipid peroxidation) and decreased levels of glutathione (GSH) and

total antioxidant capacity (TAC) (figure 2). These findings are in agreement with the generation of ROS by ZnO-NPs, leading to an overload on endogenous antioxidant defenses (Srivastava et al., 2023).
The loss of GSH, a key scavenger of ROS, further promotes neural damage, while reduced TAC indicates systemic antioxidant depletion. OMEA treatment significantly attenuated oxidative stress nearly normalizing GSH and TAC, and reducing MDA vs. ZnO-NP to near-control levels (figure 2). This robust antioxidant effect likely stems from OMAE's ability to directly scavenge ROS, enhance endogenous GSH synthesis, and up regulate antioxidant enzymes (El Riachy et al., 2021). IGF-1 also decreased oxidative stress with lesser efficacy: GSH, MDA , TAC underlining dependence on cell survival pathways rather than broad- spectrum antioxidant activity The greater efficacy of OMAE than IGF-1 in the restoration of both neurotransmitter levels and redox balance underlines the advantage of the multi-targeting nature of natural extracts against nanomaterial toxicity. Polyphenols in OMAE would have a synergistic effect on ROS suppression at three different levels: 1/ neutralization of free radicals; 2/ enhanced mitochondrial function and 3/ preservation of neurotransmitter synthesis enzymes (Panza et al., 2023).
In contrast, most of IGF-1 neuroprotection is mediated via PI3K/Akt-dependent anti-apoptotic signaling (Lee et al., 2022), which might not fully balance ZnO-NP-induced oxidative damage. These findings align with recent studies demonstrating the efficacy of plant-derived antioxidants, such as curcumin and green tea polyphenols, in mitigating nanoparticle-induced neurotoxicity (Srivastava et al., 2023).
However, OMAE's near-complete restoration of GSH and TAC (Table 2) suggests a uniquely potent antioxidant profile, possibly due to its diverse phytochemical composition. OMAE is probably potent due to its high content of polyphenols, such as rosmarinic acid and carvacrol, which directly scavenge ROS, reduce lipid peroxidation, and protect enzymatic active sites from oxidative damage (El Riachy et al., 2021). These compounds also up regulate endogenous antioxidant pathways, including nuclear factor erythroid 2-related factor 2, enhancing SOD and CAT synthesis (Panza et al., 2023). By contrast, IGF-1-a neurotrophic factor mediates enzymatic restoration through the activation of the PI3K/ Akt/ m TOR pathway, promoting neuronal survival, protein synthesis, and mitochondrial biogenesis (Lee et al., 2022). Even though IGF-1 indirectly ameliorates oxidative stress by increasing cellular resilience, its single-target mechanism could render it much less capable than the multi-targeting antioxidant action of OMAE in fully neutralizing ZnO-NP-induced enzymatic inactivation. Mechanistic Implications and Therapeutic Potential The differential efficacy of OMAE and IGF-1 addresses the importance of targeting both events of oxidative stress and cellular repair pathways for Nano toxicology. The restoring efficiency of AChE activity by OMAE, being highly crucial for the hydrolysis of acetylcholine and synaptic function, points toward additional advantages in maintaining cholinergic neurotransmission, which is mostly impaired in neurodegenerative disorders (Srivastava et al., 2023). Moreover, a more full-value restoration by OMAE of SOD and CAT activities testifies to its participation in rebalancing redox homeostasisan important mechanism participating in mitigation nanoparticle- induced neurotoxicity. These findings are in line with recent works showing the efficiency of natural antioxidants like quercetin and resveratrol in reinstating enzymatic activities impaired by metallic nanoparticles (Prakash et al., 2023). However, the superior performance of OMAE, when compared to IGF-1, underlines the therapeutic advantage of extracts rich in phytochemicals over single- agent growth factors in fighting multifaceted oxidative damage. In a nutshell, ZnO-NP exposure disturbs neurotransmitter homeostasis, which is very likely to be an effect of an oxidative stress mechanism. Bothe OMAE and IGF-1 have restorative benefits, but OMAE, being more effective, carries a big therapeutic promise for natural extracts in mitigating nanomaterial-induced neurotoxicity. Further researches will explain more on bioavailability, pharmacokinetics, long-term effects, and possible synergistic effects with other neuroprotective agents.

CONCLUSION

OMAE demonstrated preferable neuroprotective effects compared to IGF-1 in attenuating ZnO-NP-induced stress. The multifaceted antioxidant activity of OMAE makes it more potent than IGF-1 in reversing neurochemical and oxidative damage. These findings highlight the efficacy of OMAE as a natural therapeutic agent for nanoparticle-induced neurotoxicity. Further studies on the bioavailability of OMAE, synergistic action with neurotropic factors, and long-term neuroprotective efficacy should be carried out.

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