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The Ameliorative Role of Oregano aqueous extraction (*Origanum vulgare*) on Liver Enzymes, Hormones (Testosterone and Cortisol) and Antioxidant Parameters in Male Rats Exposed to Aluminum Oxide Nanoparticles

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ABSTRACT

Aluminum oxide nanoparticles Al_2O_3-NPs are extensively applied in medicine, manufacturing and consumer products. However, their bioaccumulation- particularly in the liver - induces oxidative stress, and hepatocellular damage. Chronic exposure to Al_2O_3- NPs disrupts liver function, deteriorates testosterone synthesis and reduces antioxidant defenses. Oregano *or (Origanum vulgare*) , a Mediterranean herb exhibits potent antioxidant properties, anti-inflammatory and hepatoprotective features. Regardless evidence of its therapeutic interest, limited studies inspect its role in counteracting nanoparticle-induced toxicity. Forty adult male Norwegian rats are divided into four equal groups and treated orally as follows: G1**:** control (normal saline), G2**:** Al_2O_3 NPs (50 mg/kg/day), G3: Oregano aqueous extract (200 mg/kg/day) and G4: Combination the same doses of Al_2O_3 NPs + oregano extract . The experiment lasted for 30 days, animals were sacrificed after 24 hours from the last administration , blood samples were collected to study the biochemical parameters as well as the livers and testes were removed and preserved for histopathological examination. Results : levels of liver enzymes (ALT,AST and ALP), hormones (testosterone and cortisol) and antioxidant biomarkers (SOD), (GSH), (CAT) and (MDA) were improved by oregano aqueous extract in the combination group when compared to the same parameters in Al_2O_3-NPs group as well as its ameliorative role in the histopathological sections .In conclusion Oregano extract mitigates Al_2O_3- NP toxicity through antioxidant and anti-inflammatory mechanisms, supporting its use in nanoparticle-related toxicity management .

Keywords: *Al_2O_3-NPs, Oregano, rats, GSH, cortisol.*

1. INTRODUCTION

Advancements in nanotechnology have increased exposure to engineered nanoparticles, elevating attentions about their biocompatibility. Aluminum oxide nanoparticles Al_2O_3-NPs are extensively applied in medicine, manufacturing and consumer products, due to their resistance and thermal stability. However, their high reactivity and small size enable bioaccumulation, especially in the liver, induces oxidative stress, and hepatocellular damage (Athinarayanan et al., 2020). Chronic exposure to Al_2O_3- NPs disrupts liver function, thus liver enzymes (ALT, AST, ALP) are elevated, due to accumulation of Al_2O_3 NPs in hepatic tissue generating reactive oxygen species (ROS) that damage cell membranes, mitochondria, and DNA (Rajeshkumar et al., 2018). In testes Al_2O_3 NPs causes deteriorates testosterone synthesis through testicular oxidative damage (Li et al., 2020). Aluminum ions (Al^{3+}) disrupt steroidogenesis by inhibiting 17β-hydroxysteroid dehydrogenase (17β-HSD) and cytochrome P450 enzymes in Leydig cells, reducing testosterone synthesis (Zhu et al., 2020). Concurrent oxidative stress exacerbates testicular atrophy and hormonal imbalance due to reduces antioxidant defenses. Oregano *or (Origanum vulgare*) , a Mediterranean herb (medical plant) rich in phenolic compounds (carvacrol, thymol, rosmarinic acid), exhibits potent antioxidant, anti-inflammatory and hepatoprotective features (Sharifi-Rad et al., 2021). Oregano has many Pharmacological Properties, this due to the bioactive compounds ,particularly carvacrol, which scavenge free radicals, enhance glutathione synthesis, and inhibit pro-inflammatory cytokines (Bakkali et al., 2008). Clinical trials highlight its efficacy in reducing oxidative stress markers (MDA) and improving hepatic function (Khashan et al., 2021). Regardless evidence of its therapeutic interest, limited studies inspect its role in counteracting nanoparticle-induced toxicity

and limitations include lack of histopathological data and long-term effects. This search aimed to investigates the protective role of *Origanum vulgare* (oregano) extract against aluminum oxide nanoparticle (Al_2O_3 NP)-induced hepatotoxicity, hormonal imbalance and oxidative stress in male Norwegian rat.

2. MATERIALS AND METHODS

2.1. Experimental animals , Extraction and chemical substances

Forty adult male Norwegian rats weighing (200–225 g) at age of (6-7 weeks) old were housed under standard conditions, at a temperature- controlled environment of 22-25°C and under 12 h: 12 h light/dark cycles, with ad libitum to food and water. Oregano dried leaves were bought from the market in Nasiriya city, at the beginning leaves were rinsed with distilled water to remove soil and contaminants, then air-dried in the shade at 25°C for 72 hours to prevent thermal degradation of volatile compounds. After that they were ground into a powder, then 100 g of this powder were mixed with 1 litter of distilled water (1:10 w/v) in a glass reactor. The mixture was heated to 90°C (±3°C) for 2 hours, Then the extract was filtered to remove coarse debris, and eliminate fine particulates. This study used dry powdered Al2O3-NPs (Cat No. 544833; Sigma-Aldrich, St. Louis, MO, USA).

$Al_2 O_3$ Nanoparticles (Sigma – Aldrich , < 50 nm) were suspended in saline for oral administration.

2.2. Experimental Design

Animals were divided into four equal groups**:**

G 1(n=10) : Control given (normal saline).

G 2(n=10): Al_2O_3 NPs alone (50 mg/kg/day, orally).

G 3(n=10): Oregano aqueous extract alone (200 mg/kg orally).

G 4(n=10): Combination of Al_2O_3 NPs (50 mg/kg) + oregano extract (200mg/kg)orally.

The experiment lasted for 30 days, then animals were sacrificed after 24 hours from the last administration , blood samples were collected (5 ml) and centrifuged for 10 minutes at 3000 r/minute. Sera were separated to study the biochemical parameters as well as the livers and testes were removed and preserved in formalin 10% after that they prepared for study of histopathological examination. The study was approved by the (IEC) Institutional Ethical Committee in the College of Veterinary Medicine, department of physiology, Shatrah University, Iraq, and all experimental protocols were conducted in accordance with the guidelines for the care and use of laboratory animals.

2.3. Biochemical parameters

Liver enzymes: ALT, AST, ALP measured by spectrophotometer using commercial kits.

Hormones: Testosterone and Cortisol quantified by ELISA (using assay kits obtained from Accu-Bind, Monobind, USA).

Antioxidants: Serum superoxide dismutase (SOD), reduced glutathione (GSH) , and catalase (CAT) levels and malondialdehyde (MDA) were determined by using High Performance Liquid Chromatography (HPLC) as mentioned by (Karatas, et al., (2002) and Omidi, et al., (2016)) .

2.4. Statistical Analysis

Data analysis was done by using one-way ANOVA, using SPSS 25.0. The results are expressed as mean ± SD. The significance ratios was considered at ≤ 0.05.

3. RESULTS

3.1. Liver enzymes:

Results in table.1 appeared an effective role of oregano aqueous extract as a significant (**P ≤ 0.05**) improving liver enzymes ALT, AST, and ALP activities after combined administration with Al_2O_3- NPs .

Table. 1: The effective role of oregano aqueous extract on liver enzymes of male rats after combining administration with Al_2O_3- NPs .

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
G 1: Control	35.2 ± 2.8 **c**	85.4 ± 5.9 **c**	120 ± 8.8 **c**
G 2: Al_2O_3 NPs	98.6 ± 3.4 **a**	210 ± 5.7 **a**	319 ± 9.2 **a**

G 3: Oregano	39.5 ± 1.9 **bc**	88.2 ± 3.8 **bc**	131 ± 6.5 **bc**
G 4: Al_2O_3 NPs + Oregano	42.1 ± 3.9 **b**	95.2 ± 8.5 **b**	135 ± 7.9 **b**

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

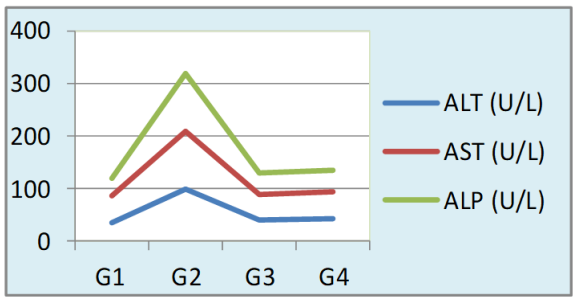


Figure. 1: The effective role of oregano aqueous extract in restoring liver enzymes to normal levels after combining administration with Al_2O_3- NPs.

3.2.Hormones:

Results in table.2 appeared an effective role of oregano aqueous extract as a significant (P ≤ 0.05) improving of (testosterone and cortisol) after combined administration with Al_2O_3- NP.

Table. 2: The effective role of oregano aqueous extract in restoring the hormones(testosterone and cortisol) to normal levels in male rats after combining administration with Al_2O_3- NPs .

Group	**Testosterone (** ng/dl**)**	**Cortisol (** ng/dl**)**
G1: Control	6.3 ± 0.1 **a**	0.66 ± 0.3 **b**
G2: Al_2O_3 NPs	1.8 ± 0.3 **c**	1.31 ±0.7 **a**
G3: Oregano	5.9 ± 0.9 **ab**	0. 64 ±0.3 **b**
G4: Al_2O_3 NPs + Oregano	5.7 ± 0.6 **b**	0.92 ±0.2 **b**

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

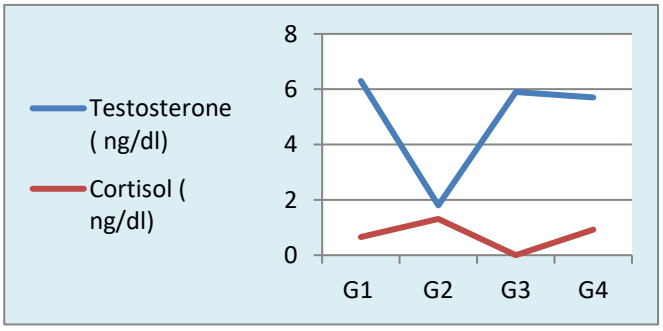


Figure. 2: The effective role of oregano aqueous extract in restoring testosterone and cortisol hormones to normal levels after combining administration with Al_2O_3- NPs.

3.3. Oxidative Stress Biomarkers

Results in table (3) appeared that the combination of oregano aqueous extract with Al_2O_3- NPs improved (SOD, GSH, CAT and MDA) levels significantly (P ≤ 0.05) when compared to these parameters in group given only Al_2O_3- NPs.

Table. 3 : (SOD, GSH, CAT and MDA) levels in plasma of control and other experimental groups.

	SOD (U/ml)	GSH (nmol/ml)	CAT(U/ml)	MDA (nmol/ml)
G1: Control	4.42 ± 0.8 **a**	0.84 ± 0.08 **a**	8.78 ± 1.6 **a**	2.12 ± 0.8 **c**
G2: Al_2O_3 NPs	1.40 ± 0.2 **c**	0.31 ± 0.22 **c**	3.10 ± 0.4 **c**	4.98 ± 1.2 **a**
G3: Oregano	4.30 ± 0.6 **ab**	0.81 ± 0.12 **a**	8.65 ± 1.3 **ab**	2.30 ± 0.9 **c**
G4: Al_2O_3 NPs + Oregano	3.98 ± 0.5 **b**	0.65 ± 0.17 **b**	7.55 ± 1.9 **b**	3.14 ± 1.1 **b**

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

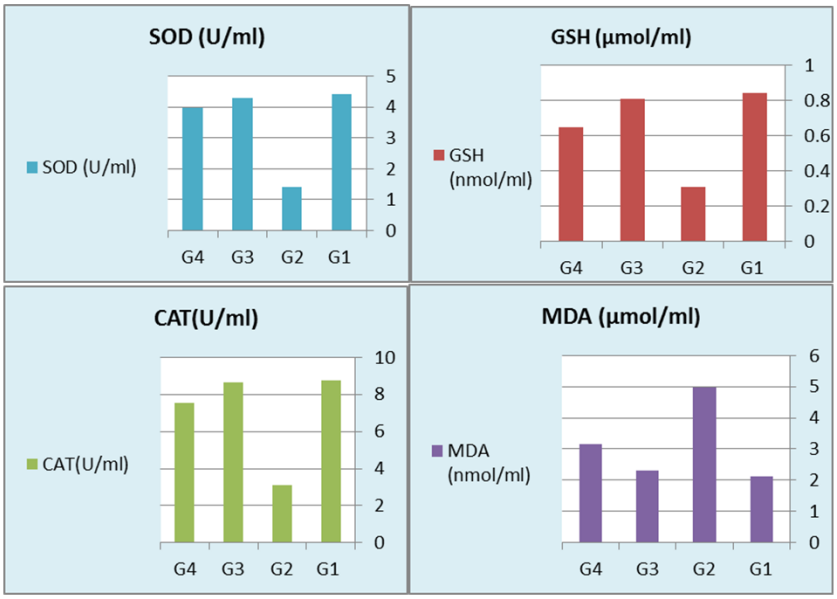


Figure 3. The effective role of oregano aqueous extract in restoring antioxidant parameters(SOD, GSH, CAT and MDA) levels significantly to normal levels after combining administration with Al_2O_3- NPs.

3.4. Histopathological examination.

- **Liver sections**

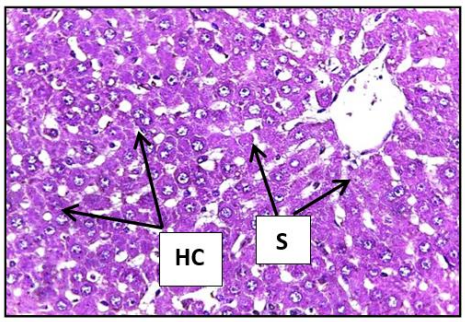


Figure.4. liver of male rat in control group showing normally formed large flattened hepatocytes (HC), with walls arranged in plates radiated toward the central vein normal sinusoids (S) architecture (H & E stain) 40 X

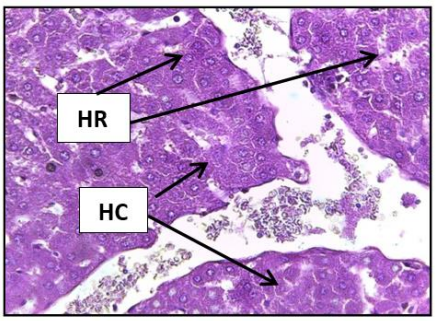


Figure.5. liver of male rat in Al2O3 group showing hepatocytes are fused with each other (HC). Not well arranged hepatic rays architectures (HR) (H & E stain) 40 X .

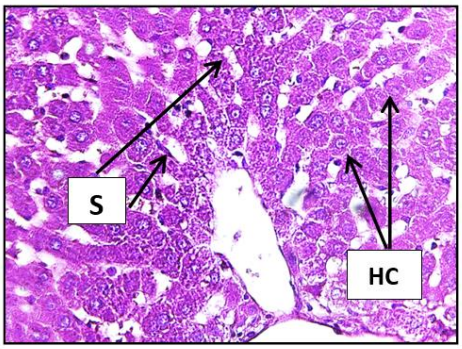


Figure.6. liver of male rat in oregano group showing normally formed large flattened hepatocytes (HC), with walls arranged in plates radiated toward the central vein normal sinusoids (S) architecture (H & E stain) 40 X

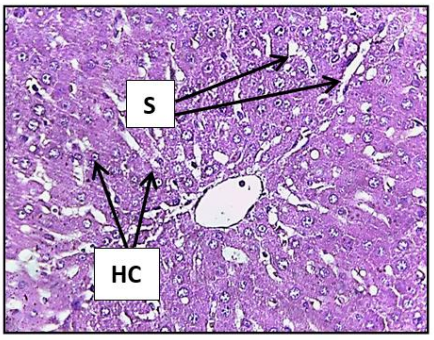
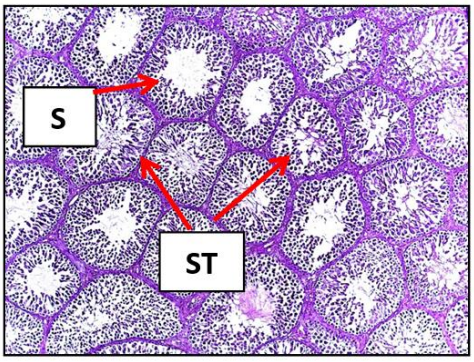
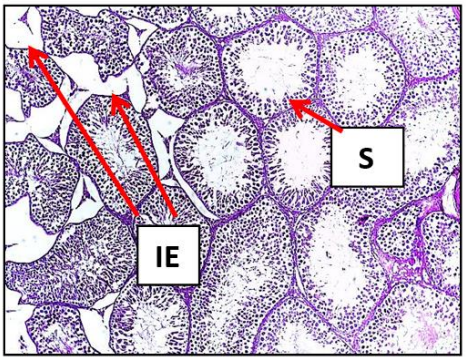


Figure.7. liver of male rat in combination group showing restoration of hepatocytes (HC), with walls arranged in plates radiated toward the central vein normal sinusoids (S) architecture (H & E stain) 40 X

- **Testes sections**



(fig. 8) Testis of male rat in control group (G1) showing normal Sertoli cells (S) normal architecture of seminiferous tubules (ST), with normal spermatogenesis stages (H & E stain) 10 X.



(fig. 9) Testis of male rat in group (G2) showing semi-normal Sertoli cells (S) with presence of an interstitial edema (IE) and semi -normal spermatogenesis stages (H & E stain) 10 X.

4. DISCUSSION

Limited studies direct the effective role of oregano aqueous extraction in moderating nanoparticle toxicity. This research connects the gap by speculating its effects on liver enzymes, stress hormones, and antioxidant pathways in Al_2O_3- NP-exposed models .

Firstly elevated levels of ALT, AST, and ALP in serum of Al_2O_3-NPs group animals significantly reflect hepatocellular injury, Al_2O_3 NPs generate reactive oxygen species (ROS), inducing lipid peroxidation, cytochrome P450 inhibition, DNA damage, and apoptosis (Abdel-Wahhab et al., 2019). Studies report elevated liver enzymes (ALT, AST) and histopathological changes in rodents exposed to Al_2O_3 NPs, indicating hepatotoxicity (Rajeshkumar et al., 2018).

In this search oregano extraction exhibited normalization of liver enzymes, that aligns with carvacrol's ability to stabilize lysosomal membranes and regenerate hepatocytes (Khalil et al., 2020). Oregano aqueous extraction showed superior efficacy, that could be due to higher phenolic bioavailability, or may be due to Carvacrol and thymol in oregano scavenge ROS, enhance Nrf2-mediated antioxidant pathways, and inhibit pro-inflammatory cytokines (TNF-α, IL-6) (Neetu and Surender. 2022).

Second, the Aluminium oxide nanomaterials have nanoparticles tend to change the production of testosterone by affecting the functionality of Leydig cells. Al3+ ions seem to have a detrimental effect on steroidogenesis due to the disruption of 17β-hydroxysteroid dehydrogenase (17β-HSD) and cytochrome P450 enzymes activity in Leydig cells, leading to lessened testosterone production (Zhu et al., 2020). Oxidative stress aggravates testicular wasting and endocrine disorders. and the increase in cortisol because of Li oxidative stress (2020). But in the combination group, oregano supplementation led to the

restoration of testosterone which is likely due to improved functioning of the Leydig cells. The increase in testosterone corresponds to a decrease in oxidative damage to Leydig cells and increased activity of other steroidogenic enzymes. Moreover, the decrease in cortisol indicates decreased stress response. This is most likely because carvacrol has metal chelating effects hence, Al3+ bioavailability is low, and so the toxicity is increased. These results support the work of authors who reported on the effectiveness of oregano in cadmium toxcicity of liver (Aydın et al., 2016). The aqueous extract of oregano also caused overproduction normalization which is a sign of reduced oxidative damage. These results are also consistent with those obtained from thyme and rosemary (Bacanlı et al., 2019).

Alongside the results of this study support the fact that Oregano alleviated oxidative stress induced by Al_2O_3- NP through the enhancement of Superoxide dismutase, Catalase, Glutathione and reduction of Malondialdehyde. Carvacrol stimulates antioxidant enzyme production by activating the Nr f2/Keap1 pathway (Sharifi- Rad et al., 2021), or perhaps it is the case that the Oregano extract reduced carbon tetrachloride toxicity in the liver of some rodents by increasing SOD and CAT activity while decreasing glutathione, which indicates some level of antioxidant defenses were present (Khalil et al., 2020). In the combination group, Oregano's phenolic compounds seem to have scavenged Al_2O_3 NP induced ROS, leading to reduced lipid peroxidation MDA levels and increased SOD and CAT activity. Histopathological results of this study were similar to those when oregano extract was used to treat cadmium induced hepatotoxicity in rats (Mohammad S. et al., (2012). To my knowledge, this is the only paper to demonstrate the dual action of oregano in the alleviation of both liver and testicular damage from Al_2O_3 NPs.

5. CONCLUSION

Origanum vulgare aqueous extract diminish Al_2O_3 fertilized NP hepatotoxicity, testosterone level reduction, and lipid peroxidation via reactive oxygen species depression, inflammation inhibition, and enzyme modulation. This was done using a method of nanoparticles-informed medicaments. for the incorporation into nutrition plans to reduce the impact of environmental nanoparticles. Later research can analyze specific dosing and how to apply it clinically.

6. ACKNOWLEDGMENT

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