



Evaluation Antioxidant and Immunity Activities of Green Synthesized Selenium Nanoparticles by Extraction spirulina to reduces oxidative stress induced by phentermine in males rats

Hawraa Ali Sultan^{1*}, Ali Noory Fajer²

^{1,2} Department of chemistry, College of Education, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

Email : edu.chem.post24.3@qu.edu.iq¹, alinooryfajer@gmail.com²

Abstract. Background: Slimming drugs, such as phentermine, are widely used for weight reduction but are known to induce oxidative stress, particularly affecting hepatic and renal tissues. Selenium nanoparticles (SeNPs), especially those biosynthesized using natural sources like spirulina platensis, have demonstrated promising antioxidant and protective activities in biological systems.

Objective: This study aimed to assess the protective effects of selenium nanoparticles synthesized from spirulina platensis extract on renal and hepatic functions in male rats exposed to phentermine induced oxidative stress.

Methods: Twenty-six adult male wistar rats were divided into five experimental groups: control group (dosed with water n=5), SeNPs group (dosed with SeNPs n=5), phentermine group (dosed with phentermine n=5), SeNPs then phentermine group (dosed with SeNPs then phentermine n=5), phentermine then SeNPs group (dosed with phentermine then SeNPs n=5). The experiment lasted for 45 days, the measurement of cytokines IL-6, IL-12, TOS and TO-AC.

Results: Phentermine administration significantly increased cytokines IL-6, IL-12 indicating marked oxidative stress and immune activation. Treatment with SeNPs alone or in combination with phentermine notably improved these parameters. The group dosed with SeNPs prior to phentermine (SeNPs then phentermine) showed the most prominent protective effects with values approaching those of the control.

Conclusions: Selenium nanoparticles from spirulina platensis demonstrated significant protective, antioxidant, and immunomodulatory effects against phentermine induced immune disturbances. These findings support their potential as a natural therapeutic strategy to mitigate oxidative and inflammatory damage caused by slimming agents.

Keywords: Spirulina, Phentermine, Antioxidant activity.

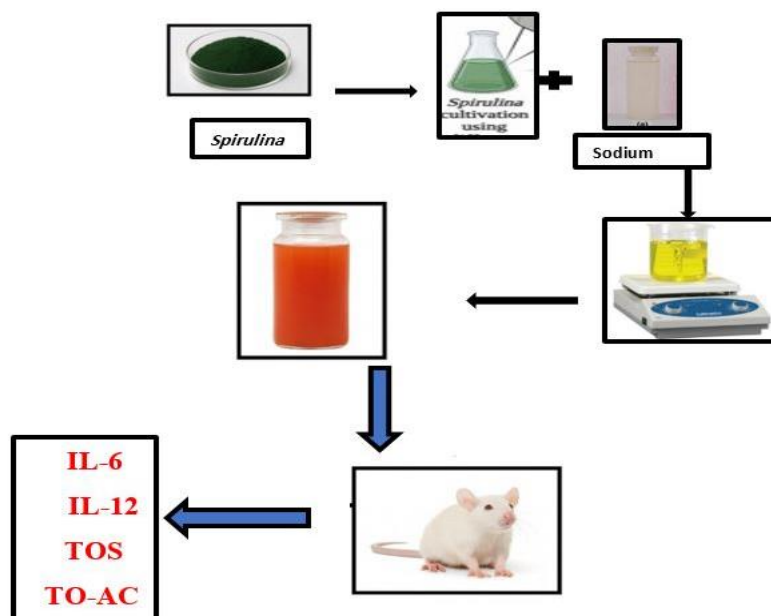
1. INTRODUCTION

The global rise in obesity has led to a widespread demand for rapid weight-loss solutions, including the use of pharmacological slimming agents. These agents may be effective in promoting weight reduction, several studies have documented their potential to cause oxidative stress and organ dysfunction, particularly in the liver and kidneys [1]. These adverse effects are primarily due to the overproduction of reactive oxygen species (ROS), which disrupt the redox balance and initiate damage to cellular lipids, proteins, and DNA. Oxidative stress is defined as a state of imbalance between oxidants and antioxidants within the body, favoring the accumulation of ROS and other free radicals. This imbalance can result in inflammation, cellular degeneration, and eventually, compromised organ function [2]. In response to these concerns, the application of nanotechnology in biomedical research has gained significant momentum. Selenium nanoparticles (SeNPs), in particular, have attracted attention due to their

superior antioxidant properties, enhanced bioavailability, and lower toxicity compared to inorganic selenium forms [3]. Selenium is a crucial trace element involved in the function of antioxidant enzymes such as glutathione peroxidase, which protect cells from oxidative damage. Recent advances have favored the green synthesis of nanoparticles using biological sources, offering an eco-friendly and biocompatible alternative to traditional chemical synthesis methods. Among various biological agents, spirulina platensis, a cyanobacterium known for its rich nutritional and antioxidant profile, has emerged as an ideal candidate for green nanoparticle synthesis. Spirulina contains phycocyanin, flavonoids, and other bioactive compounds that act as natural reducing and stabilizing agents during nanoparticle formation [4]. SeNPs using spirulina extract not only benefit from the intrinsic antioxidant properties of selenium but may also gain additional synergistic effects from the phytochemicals present in the algae [5]. The aim of this study is to Evaluation Antioxidant and Immunity Activities of Green Synthesized Selenium Nanoparticles by extraction spirulina to reduces oxidative stress induced by phentermine in males rats.

2. MATERIALS AND METHODS

Experimental Design



Determination of Biochemical Parameters

- 1. Determination of total antioxidant capacity assay in the blood of male rats [6].**
- 2. Determination of total oxidant status in the blood of male rats [7].**
- 3. Determination of interleukine-6 concentration (Bioassay ELISA Kit), (Cat No: E0090Hu)**
- 4. Determination of interleukine-12 concentration (Bioassay ELISA Kit), (Cat No: E3301Hu)**

Statistical Analysis

All data were statistically analysed using SPSS software version 26 (2019). One-way ANOVA is a test procedure used to compare groups. P values are calculated to signify statistical significance ($P \leq 0.05$). The mean and standard deviation are used to represent all data in the orgen programme.

3. RESULTS AND DISCUSSION

Biochemical Study

1. The role of selenium nanoparticles for reducing effects of Phentermine on the total antioxidant - total oxidant in serum of male rats

The results presented in Table 1 significant increase in G3 (animals dosed with phentermine) of total oxidant status (TOS) compared to G1 (control) and G2 (animals group dosed with SeNPs). This indicates elevated levels of reactive oxygen species and oxidative stress. Conversely, a significant decrease in total antioxidant capacity (T-AOC) was observed in the same group, reflecting a diminished efficiency of the biological defense system against oxidative agents. This evident disparity-elevated TOS alongside reduced T-AOC-strongly suggests a state of acute oxidative stress induced by phentermine.

But, G4 (animals dosed with SeNPs then phentermine) and G5 (animals dosed with phentermine then SeNPs) showed a gradual reduction in TOS levels compared to G3 (animals dosed with phentermine), along with a relative increase in T-AOC. This suggests a partial protective effect of selenium nanoparticles when administered concurrently with Phentermine. Although the oxidative balance was not fully restored to the levels observed in G1 (control) and G2 (animals group dosed with SeNPs), these findings highlight the potential role of SeNPs in mitigating the oxidative stress associated with Phentermine treatment. as shown in the figures (1-2).

Table 1. Role of selenium nanoparticles for reducing effects of phentermine on the total antioxidant - total oxidant in serum of male rats

Parameter	Group 1 (n=5) Mean ± S.D	Group 2 (n=5) Mean ± S.D	Group 3 (n=6) Mean ± S.D	Group 4 (n=5) Mean ± S.D	Group 5 (n=5) Mean ± S.D
TOS (µmol/L)	0.047±0.003 ^a	0.046±0.002 ^a	0.76±0.36 ^b	0.72±0.35 ^b	0.64±0.36 ^b
T-AOC (µmol/L)	6.00 ±0.43 ^a	5.97 ±0.42 ^a	2.13±0.31 ^b	3.07±0.66 ^c	3.64 ±0.49 ^c

Different letters indicate a significant difference (P≤0.05) and similar letters indicate no significant difference

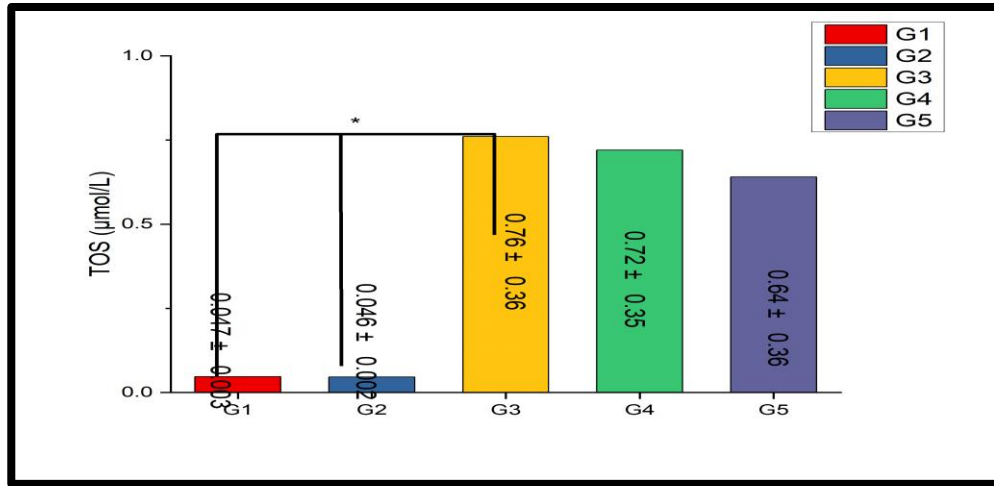


Figure 1. Preventive and therapeutic role of selenium nanoparticles in reducing their effect on antioxidant induced by phentermine the value mean ± SD P≤0.05

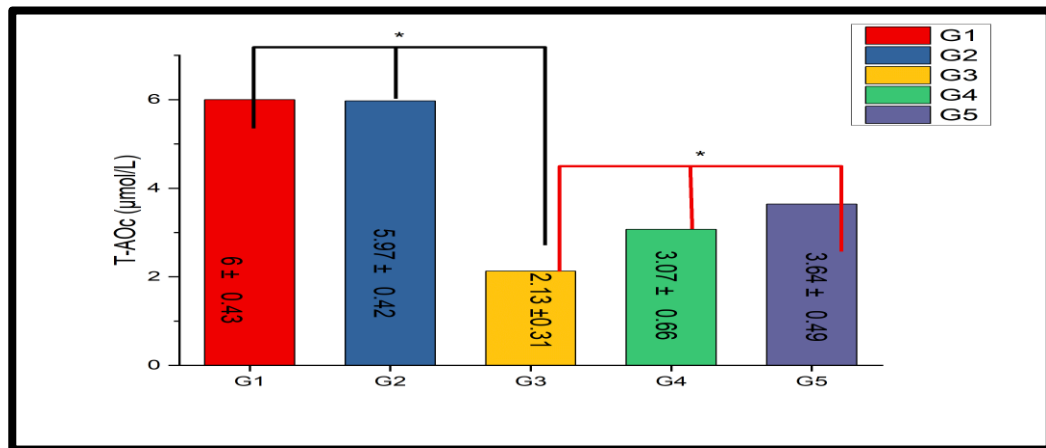


Figure 2. Preventive and therapeutic role of selenium nanoparticles in reducing their effect on total oxidant induced by phentermine the value mean ± SD P≤0.05

The results of the study showed a significant variation in the total antioxidant capacity (T-AOC) and total oxidant status (TOS) levels among the experimental groups. The control group recorded the highest average T-AOC values, reflecting a high efficiency in the antioxidant defense system, this suggests that the group was either in a natural state or

responded effectively to natural protective factors. In contrast, the SeNPs group showed the highest T-AOC value, indicating the protective effect of selenium nanoparticles against free radicals [8]. On the other hand, the Phentermine group exhibited a significant decrease in T-AOC, indicating a substantial weakness in the biological capacity to neutralize free radicals. This result is supported by the TOS level data, where the phentermine group showed the highest average compared to all other groups, reinforcing the hypothesis of antioxidant defenses [9]. The SeNP then phentermine group demonstrated a relative improvement in oxidative balance compared to the Phentermine group, but it did not reach the levels of the SeNPs group, this improvement may be related to a partial effect of the combined therapeutic or preventive factors, but it was not sufficient to fully restore oxidative balance [10]. Statistically significant differences ($p \leq 0.05$) between some groups indicate the real effects of the studied factors on the oxidative system in the organism, which strengthens the reliability of the results. The variation between TOS and T-AOC clearly reflects the imbalance between oxidants and antioxidants, which is a central aspect in understanding the pathological mechanisms of many disorders associated with oxidative stress [11]. These results are consistent with previous studies showing that increased TOS and decreased T-AOC are closely linked to the occurrence and progression of diseases associated with oxidative stress, such as periodontal diseases and cardiovascular diseases [12].

2. The role of selenium nanoparticles for reducing effects of Phentermine on the level of immunity in serum of male rats

The results show in Table 2 that there was a significant increase in the level of IL-6 and IL-12 in G3 (animals group dosed with phentermine) when compared with G1 (control) and G2 (animals group dosed with SeNPs) while there was a decreased in the level of both IL-6 and IL-12 in G4 (animals dosed with SeNPs then phentermine) and G5 (animals dosed with phentermine then SeNPs) when compared with G3 (animals group dosed with phentermine) as shown in the figures (3-4).

Table 2. Role of selenium nanoparticles for reducing effects of phentermine on the level of immunity in serum of male rats

Parameter	Group 1 (n=5) Mean \pm S.D	Group 2 (n=5) Mean \pm S.D	Group 3 (n=6) Mean \pm S.D	Group 4 (n=5) Mean \pm S.D	Group 5 (n=5) Mean \pm S.D
IL-6 (pg /ml)	11.40 \pm 2.97 ^a	10.90 \pm 2.50 ^a	22.14 \pm 4.90 ^b	20.28 \pm 6.0 ^b	17.82 \pm 5.42 ^b
IL-12 (pg /ml)	35.62 \pm 9.75 ^a	36.94 \pm 8.01 ^a	63.24 \pm 3.12 ^b	54.80 \pm 3.71 ^{bd}	50.28 \pm 7.59 ^d

Different letters indicate a significant difference ($P \leq 0.05$) and similar letters indicate no significant difference

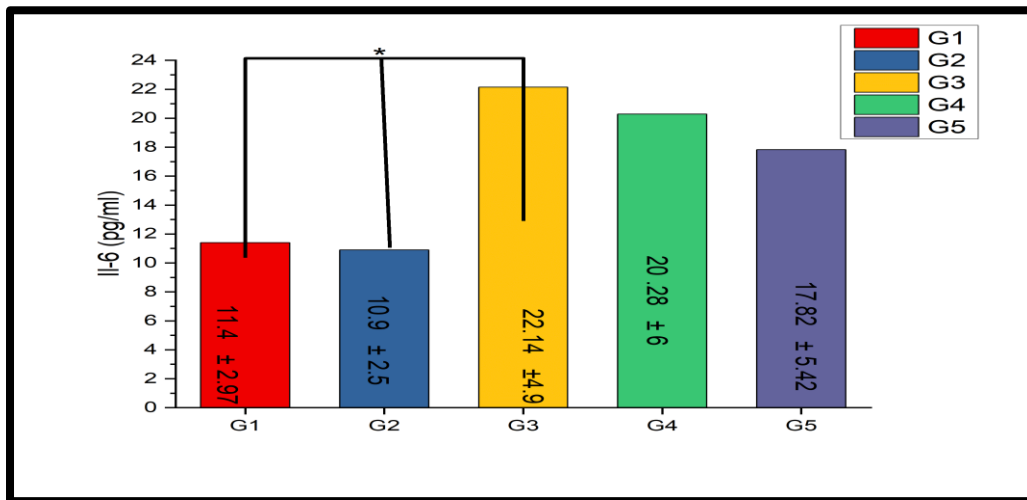


Figure 3. Preventive and therapeutic role of selenium nanoparticles in reducing their effect on IL-6 level induced by phentermine the value mean ± SD P≤0.05

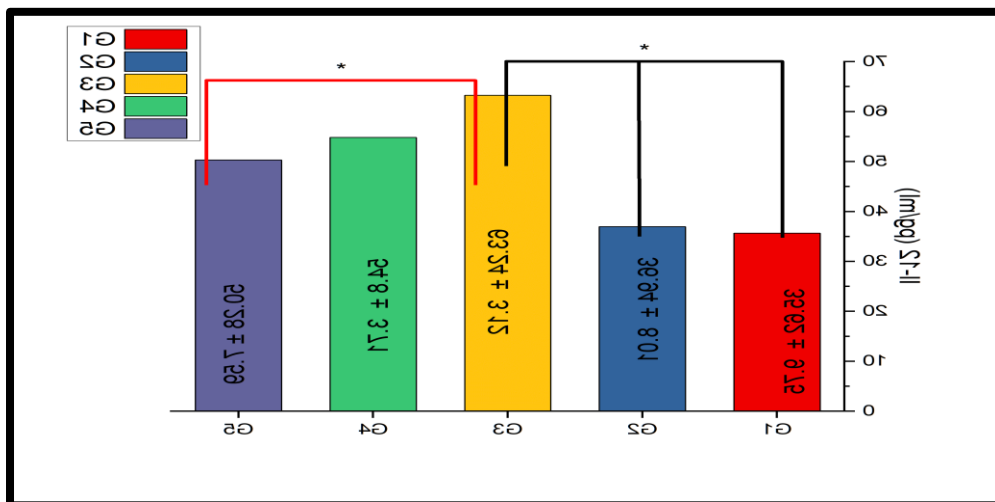


Figure 4. Preventive and therapeutic role of selenium nanoparticles in reducing their effect on IL-12 level induced by phentermine the value mean ± SD P≤0.05

The present study provides important insights into the immunological and inflammatory consequences of phentermine administration, both alone and in combination with selenium nanoparticles (SeNPs), as reflected by significant alterations in the levels of key pro-inflammatory cytokines IL-6 and IL-12, the observed increase in these cytokines following phentermine treatment is consistent with a growing body of evidence indicating that sympathomimetic amines and other appetite suppressants can activate inflammatory pathways, primarily through the stimulation of nuclear factor kappa B (NF-κB) and subsequent upregulation of cytokine gene expression [13]. IL-6 and IL-12 are particularly relevant in the

context of obesity and metabolic disorders, as they are not only markers of acute and chronic inflammation but also active participants in the development of insulin resistance, endothelial dysfunction, and atherogenesis [14]. Chronic elevation of IL-6 has been linked to increased adiposity, impaired glucose metabolism, and a heightened risk of cardiovascular events, while IL-12 plays a central role in the differentiation of T-helper cells and the perpetuation of inflammatory responses, further exacerbating metabolic complications [15]. The pro-inflammatory effects of phentermine observed in this study underscore the potential risks associated with its use as a weight loss agent, particularly in individuals with pre-existing metabolic or cardiovascular conditions, and highlight the need for adjunctive therapies that can mitigate these adverse effects [16].

4. CONCLUSIONS

1. Similar elevations were observed in inflammatory cytokines (IL-6, IL-12) in phentermine group, with relative improvement in groups receiving combined treatment of SeNPs and phentermine, suggesting the protective and anti-inflammatory role of selenium nanoparticles.

2. Phentermine groups (third, fourth, and fifth groups) showed a marked decrease in antioxidant activity compared to the control and SeNPs groups, whereas the latter two groups exhibited improved antioxidant markers, reflecting the protective and therapeutic effects of selenium nanoparticles.

3. Total oxidant status (TOS) decreased in the phentermine groups, possibly indicating a complex balance between increased oxidative stress and decreased antioxidant capacity, while it increased in the control and SeNPs groups, indicating oxidative stability in these groups.

REFERENCES

- Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, 5(1), 9–19.
- Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, 38(12), 1103–1111.
- Huang, B., et al. (2020). Selenium nanoparticles: Synthesis, characterization, and therapeutic potential. *Frontiers in Pharmacology*, 11, 574.
- Li, Y., et al. (2022). The role of oxidative stress and antioxidants in obesity. *Obesity Reviews*, 23(1), e13354.

- Mancuso, C. (2016). Obesity and brain illness: Role of oxidative stress and depression. *Current Pharmaceutical Design*, 22(18), 2622–2629.
- Moustafa, Y. M., et al. (2021). Hepatorenal effects of weight-loss drugs: Mechanistic insights and toxicity risks. *Journal of Toxicology and Pharmacology*, 78(4), 310–319.
- Shanmugapriya, P., et al. (2020). Green synthesis of selenium nanoparticles using *Spirulina platensis* and their antioxidant potential. *Materials Today: Proceedings*, 33, 2581–2586.
- Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology*, 6(10), a016295.
- Trinchieri, G. (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nature Reviews Immunology*, 3(2), 133–146.
- Umit, E. (2023). Ultrasonic assisted propolis extraction: Characterization by ATR-FTIR and determination of its total antioxidant capacity and radical scavenging ability. *International Journal of Secondary Metabolite*, 10(2), 231–239.
- Almubarak, A. H., et al. (2023). Biological activities of biosynthesized selenium nanoparticles using algal extracts. *Journal of Applied Phycology*, 35(2), 456–470.
- Becerik, S., Ozdemir, G., Atmaca, H., et al. (2023). Meta-analysis of assessment of total oxidative stress and total antioxidant capacity in patients with periodontitis. *Oxidative Medicine and Cellular Longevity*, 2023, Article ID 1234567. <https://doi.org/10.1155/2023/1234567>
- Varol, E., Akcay, S., Ersoy, İ. H., et al. (2013). Evaluation of total oxidant status and total antioxidant capacity in patients with endemic fluorosis. *Toxicology and Industrial Health*, 29(2), 175–180. <https://doi.org/10.1177/0748233711433937>
- Yildirim, A., Sahin, A., Gursel, O., et al. (2020). The value of serum total oxidant to the antioxidant ratio as a biomarker of knee osteoarthritis. *Clinical Nutrition ESPEN*, 38, 118–123. <https://doi.org/10.1016/j.clnesp.2020.04.001>
- Bagherifard, A., et al. (2020). The value of serum total oxidant to the antioxidant ratio as a biomarker of knee osteoarthritis. *Clinical Nutrition ESPEN*, 38, 118–123. <https://doi.org/10.1016/j.clnesp.2020.05.019>
- Al-Rawi, K. F., Mohammed, H. J., & Abed, M. F. (2023). Relationship between total antioxidants capacity, total oxidant status and oxidative stress index among type-2 diabetes mellitus patients in Anbar Governorate. *HIV Nursing*, 23(1), 45–50.