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# POLYSTYRENE NANOPARTICLES' IMPACT ON LIPID PROFILE-TESTOSTERONE RELATIONSHIP IN TESTICULAR TOXICITY: NIGELLA SATIVA RESTORATION

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#### **ABSTRACT**

The study investigated the alteration inflicted by PS-NP on the lipid profile and its association with testosterone release, as well as the potential of *Nigella sativa* oil, a natural and readily accessible medicine, to mitigate this damage. In this regard, twenty male rats were randomly assigned into four groups as follows: the control group, the NSO group (500 mg/kg body weight of organic *Nigella sativa* oil), the PS-NP group (10 mg/kg body weight of polystyrene nanoparticles), and the NSO+PSNP group (500 mg/kg body weight of *Nigella sativa* oil, followed by 10 mg/kg body weight of polystyrene nanoparticles). Rats received oral doses by gavage three times each week for 60 days. The PSNP group exhibited increased cholesterol and triglyceride levels, coupled with decreased HDL and testosterone levels, and it showed cytoplasmic vacuolation, degeneration of spermatogenic cells, and vascular congestion in testicular tissue. Conversely, the group NSO+PSNP exhibited reduced cholesterol and triglyceride levels, with normal HDL and testosterone levels. The testicular tissue showed minor pathological alterations, including slightly congested blood vessels. In conclusion, *Nigella sativa* oil showed substantial effectiveness in mitigating the detrimental effects induced by PS-NP on the male hormone connected with lipid metabolic alteration.

**Key words**: Polystyrene nanoplastic; *Nigella sativa*; cholesterol; triglycerides; testosterone.

#### INTRODUCTION

Nanoplastics (NPs) pose a significant threat to human health owing to their prolonged environmental persistence and their ability to absorb various hazardous contaminants, including heavy

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metals from the environment (Khedre et al., 2023). In addition to their ability to enter the body through multiple routes, including ingestion, inhalation, and dermal contact (Allouzi et al., 2021; Alaraby et al., 2022). Due to their small size, nanoplastics (NPs) can penetrate biological barriers, including cellular membranes, the blood-brain barrier, and even the placental barrier, allowing them to reach critical organs and tissues (DeLoid et al., 2024; Yang et al., 2024). Polystyrene nanoparticle (PS-NPs) is a common type of nano-plastic, resistant to degradation (Yang et al., 2022), and may

enter the body or convey contaminants, causing health hazards (Senathirajah et al., 2020). PS-NPs damage the lungs and may induce chronic respiratory illnesses (Zha et al., 2023), while in the neurological system, they alter cognition, memory, neurotransmitter function. possibly accelerating neurodegenerative diseases like Alzheimer's and Parkinson's (Jin et al., 2022; Wang et al., 2023). They also accumulate in the kidneys, causing inflammation and fibrosis damage (Wang et al., 2020; Shen et al., 2023). PS-NPs also reduce fertility (Huang et al., 2022), impair placental function (Hu et al., 2021), and increase fetal development risks (He and Yin, 2023). PS-NP was reported to cause digestive issues, such as changes in gut flora and metabolic disorders (Lu et al., 2018). PS-NP exposure has been shown to impair cellular lipid metabolism, particularly in human macrophages, where it promotes the accumulation of lipid droplets within the and fosters macrophage cvtoplasm differentiation into foam cells (Florance et al., 2022). PS-NPs disrupted the bloodtesticular barrier (Jin et al., 2020), inducing apoptosis in testicular tissues (Hou et al., 2020), disrupting male hormonal balance, which is essential for spermatogenesis (Sui et al., 2023). Currently, investigations are exploring potential mitigation treatments, including the use of probiotics (Li et al., 2024). Nigella sativa in research demonstrated its potential ameliorative effects on male reproductive-induced toxicity through its remarkable antiinflammatory and antioxidant properties (Shahid et al., 2021). This study investigates the potential of Nigella sativa oil extract, a natural and widely available remedy with numerous health benefits, in restoring damage caused by PS-NP in the male reproductive system related to lipid metabolic alteration.

### **MATERIALS AND METHODS**

#### **Ethical Considerations**

The ethical committee of the Veterinary Medicine Faculty, Sohag University,

authorized this experimental protocol and procedures. The ethical approval number is Soh. Un. Vet/00064D.

#### 1- Chemicals

Polystyrene nanoparticles were purchased from Nano-gate, Egypt. *Nigella Sativa* oil was sourced from a local herbarium and oil mill. Spectrum Diagnostics, Egypt, supplied kits for triglycerides, cholesterol, and high-density lipoproteins (HDL). The Testosterone Eliza kit was obtained from Calbiotech, USA.

#### 2- Animals

Twenty male Sprague Dawley rats, with an average weight of (170±5 g), were allowed a 10-day acclimatization period before the experiment. Rats were housed in stainless steel cages padded with sawdust and had unrestricted access to water and rat feeding pellets. Maintained under controlled conditions of temperature 22±3 °C, relative humidity 45–60%, and a 12-hour light-dark cycle.

# 3- Experimental design

Rats were grouped (5 per group) randomly as follows: Control group (received distilled water), NSO group (500 mg/kg BW of organic Nigella sativa oil), PSNP (10 mg/kg BW of polystyrene nanoparticles), NSO+ PSNP group (received 500 mg/kg BW of Nigella sativa oil followed by 10 mg/kg BW of polystyrene nanoparticles). Rats were administered the doses orally through gavage three times weekly for 60 days. Animals were euthanized using diethyl ether. Blood samples were obtained by cardiac puncture; EDTA Vacutainer tubes were used for whole blood samples, whilst plain tubes were used for serum samples. Centrifugation was performed at 3000 rpm for 10 minutes to collect plasma and serum. Plasma was preserved in Eppendorf tubes and maintained at -80°C until hormonal analysis. Serum samples were utilized for analysis of triglycerides, cholesterol. and HDL. Testes and epididymides were collected and weighed.

The testis was immersed in a Formoalcohol solution for histopathological examination.

# 4- Body and testicular weight

The animals' initial body weight was documented at the commencement of the study, and the final body weight was recorded just before euthanasia. The absolute Cauda-epididymis and testicular weights were documented at slaughter, and the relative organ weights were calculated relative to body weight.

# 5- Lipid profile

Total cholesterol (TC) was measured using the technique established by Ellefson and Caraway (1976). High-density lipoprotein cholesterol (HDL-C) was quantified according to Warnick and Wood (1995). Triglyceride (TG) was measured methodologically by Bucolo and David (1973) in serum.

# 6. Analysis of the Testosterone hormone

Testosterone levels were assessed in plasma using a commercial kit that employed a solid-phase competitive ELISA method (Chen *et al.*, 1991).

# 7- Histopathology

The testes were fixed in neutral formalin buffer (10%). Subsequently, the tissues were dehydrated and processed. The processed tissues were embedded in paraffin blocks, sectioned, and stained with hematoxylin and eosin (H&E), examined under an Olympus CX31 Light microscope with an Olympus C-5060 digital camera.

#### 8- Statistical analysis

Data were statistically analyzed using SPSS software version 28, employing one-way ANOVA with Dunnett's *post hoc* test. Differences in data were considered significant if  $P \le 0.05$ , and data were presented as mean  $\pm$  SE (Hansen, 2005).

#### RESULTS

# 1- Body weight and organ coefficient

The analysis indicated no statistically significant differences in the body weights of the rats across all groups during the experiment. Additionally, the results showed no significant variations in the Cauda-epididymis weight and testicular weight of male rats, both in absolute (g) and relative (%) terms, among all groups throughout the experiment (Table 1)

**Table 1:** Impact of PSNP and NSO on body weight, relative and absolute testicular, and epididymal weights in male rats.

Tissue		Control	NSO	PSNP	NSO+PSNP		
Right Testis	Absolute	$1.47 \pm 0.08$	$1.42 \pm 0.04$	$1.59 \pm 0.05$	$1.48 \pm 0.05$		
	Relative	$0.53 \pm 0.03$	$0.52 \pm 0.01$	$0.59 \pm 0.03$	$0.56 \pm 0.02$		
Left Testis	Absolute	$1.49 \pm 0.08$	$1.42 \pm 0.03$	$1.57 \pm 0.04$	$1.51 \pm 0.08$		
	Relative	$0.54 \pm 0.03$	$0.52 \pm 0.02$	$0.58 \pm 0.02$	$0.57 \pm 0.03$		
Right Cauda	Absolute	$0.20\pm0.02$	$0.17 \pm 0.01$	$0.19 \pm 0.01$	$0.17 \pm 0.01$		
	Relative	$0.07 \pm 0.01$	$0.06\pm0.00$	$0.07\pm0.00$	$0.06\pm0.00$		
Left Cauda	Absolute	$0.20 \pm 0.02$	$0.16 \pm 0.01$	$0.19 \pm 0.01$	$0.17 \pm 0.02$		
	Relative	$0.07 \pm 0.01$	$0.06\pm0.00$	$0.07\pm0.00$	$0.06\pm0.00$		
Body weight		274.33±3.17	274±5.81	265±7.25	271.8±7.65		
Data represented as mean $\pm$ S.E.							

#### 2- Lipid Panel analysis

Only the PSNP-treated group exhibited a significant increase in cholesterol levels. The NSO+PSNP group experienced a notable decline and a significant recovery difference in comparison with the PSNP-treated group. The PSNP group demonstrated a significant decrease in HDL levels compared to the other groups. The

NSO+PSNP group exhibited a significant increase in HDL levels compared to the PSNP group. Regarding triglycerides, both the PSNP and NSO+PSNP groups exhibited elevated TG levels compared to the control group; however, the NSO+PSNP group demonstrated more significantly recovered levels than the PSNP group (Table 2).

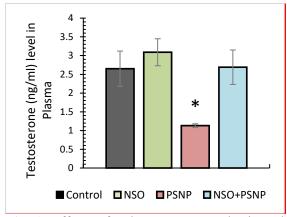
**Table 2:** Effects of NSO and PSNP on serum cholesterol, HDL, and triglyceride levels in male rats.

	Control	NSO	PSNP	NSO+PSNP
Cholesterol	$84.75\pm0.61$	$79.08 \pm 3.48$	$109.93 \pm 2.05^{ab}$	$76.21\pm0.45^{c}$
HDL	$18.43 \pm 0.83$	$20.52 \pm 1.19$	$12.16\pm0.83^{abd}$	$19.33 \pm 1.27^{c}$
Triglycerides	$26.98 \pm 1.24$	$22.82 \pm 0.94$	$44.21 \pm 0.41^{abd}$	$32.72 \pm 0.96^{c}$

Data represented as mean  $\pm$  SE (N=5). Means with Superscripts a,b,c, and d denote significant differences at P< 0.05 from Control, NSO, PSNP, and NSO+PSNP, respectively. Groups lacking superscripts are similar to the reference group.

# 3- Plasma testosterone hormone analysis

The PSNP group showed a significant reduction in plasma testosterone levels as compared to the control and NSO groups. The mitigation group (NSO+PSNP) demonstrated significant restoration in testosterone levels as compared to the PSNP group. The NSO group exhibited a non-significant change in the levels of testosterone (Fig. 1).



**Fig. 1:** Effects of Polystyrene Nanoplastic and *Nigella sativa* oil on testosterone (ng/ml) level in the plasma of male rats.

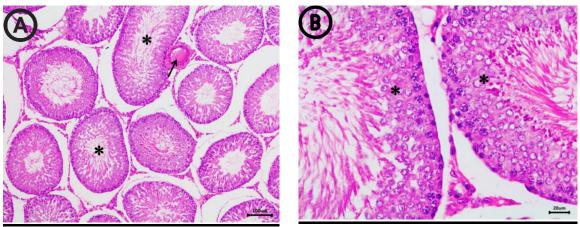
# 4 – Histopathology

Histopathological examination of testes in control and NSO groups showed normal histological architecture of testes in the two months of exposure. As for the NSO+PSNP group, testicular tissue appeared normal with mild pathological changes, such as mildly congested blood vessels (Fig. 2. A, B). Meanwhile, the seminiferous tubules in the PSNP group showed cytoplasmic vaculation and degeneration of spermatogonic cells with congestion of blood vessels (Fig. 3. A, B).

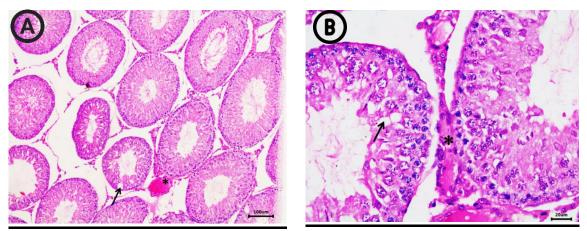
## **DISCUSSION**

In our investigation, the body weight of rats, together with the relative and absolute weights of the testes and epididymis, exhibited no significant changes, suggesting no detrimental effects from PSNP treatment. Despite PSNP exhibiting histological changes in the testicles, these modifications seemingly were not enough to affect their weight. Rafiee *et al.* (2017) reported no alterations in the body weights of rats exposed to polystyrene nanoparticles for five weeks, corroborating our findings.

This study demonstrated that the PSNP-treated group exhibited elevated cholesterol and triglyceride levels (bad fat), alongside reduced testosterone and HDL levels.



**Fig. 2:** Testicular histology of the NSO+PSNP group after 2 months showing: Normal seminiferous tubules (asterisks) and mildly congested blood vessels (arrow) in otherwise largely normal tissue architecture A) Low power, B) High power. H&E.



**Fig. 3**: Histopathological alterations in testicular tissue of the PSNP group after 2-month exposure showing cytoplasmic vaculation & degeneration of spermatogonic cells (arrow), congestion of blood vessels (asterisks). A) Low power, B) High power. H and E

In contrast, the group co-exposed to polystyrene nanoparticles and Nigella sativa oil maintained lower cholesterol and triglyceride levels, as well as normal HDL (good fat) and testosterone levels. The relationship between testosterone hormone and lipid profile levels is complex. Cholesterol serves as an essential component for Leydig cells in the synthesis of testosterone (Tyagi et al., 2017). However, some studies indicate no association between testosterone and serum cholesterol levels (Gomes et al., 2023), other studies suggested while that testosterone treatment may reduce cholesterol levels (Yassin et al., 2025). HDL, which is considered good cholesterol, participates in testosterone synthesis through carrying cholesterol into Leydig cells (Thorngate & Williams, 2004) or by acting as an anti-inflammatory in the testicular tissue (Rovira-Llopis et al., 2017). Clinical studies have linked increased testosterone production to a higher ratio of HDL to non-HDL cholesterol (Mei et al., 2025). Research studies indicated that elevated triglyceride levels contributed to decreased testosterone production by increasing inflammatory cytokines, which adversely affect Leydig cells' function. This may also result from triglycerides and subsequently increased reactive oxygen species (ROS), resulting in damage to testicular tissue, including Leydig cells, or by causing endothelial dysfunction that impairs nutrient flow to the testicular tissue (Jarvis et al., 2020; Grandys et al., 2021). These studies are inconsistent with our findings of elevated cholesterol and triglyceride levels accompanied by a decline in testosterone concentration in the PSNP-treated group. Prior research explained that polystyrene nanoparticles induce lipid disorders by influencing the activity of lipid metabolism-related enzymes and their gene expression, while also causing fluctuations in triglyceride levels and HDL, contingent upon PSNP concentration (Li *et al.*, 2023; Yu *et al.*, 2024).

Nigella sativa oil boosts testosterone owing to its benefits. It has been shown to enhance lipid metabolism by reducing cholesterol and triglyceride levels while increasing HDL levels, thereby promoting testosterone production (Bashandy et al., Rashidmayvan et al., 2019). It possesses anti-inflammatory and antioxidant properties that safeguard against testicular damage (Almujaydil et al., 2023). The histopathological investigation indicated that the testicular tissue of rats exposed to polystyrene nanoparticles had histological alterations, indicative of toxic damage, two months after exposure. The alterations included cytoplasmic vacuoleation, disruption of spermatogenic cells, and degeneration. Meanwhile, the testicular tissue of rats subjected to Nigella sativa oil in conjunction with PSNP exhibited little damage and enhanced histological structure. suggesting that N.S. mitigated the detrimental effectively histological changes in the testis induced by Cytoplasmic PSNP. vacuolation spermatogenic cell degeneration may be regarded as chemically induced damage, steroidogenesis likely indicative of disruption.

Cytoplasmic vacuolation is considered an indicator of blood-testicular barrier disruption (Johnson, 2014). Polystyrene microplastics have been shown to compromise the integrity of the blood-testis barrier via reactive oxygen species (Wei *et al.*, 2021). In harmony with our study, PSNP can build up in the testes, cause

oxidative stress, alter gene expression linked to inflammation and apoptosis, and impair energy metabolism, all of which can lead to damage to the microstructure and functioning of the testicles (Xu et al., 2023). In line with our findings, oral gavage of PS-NPs in mice for 60 days caused morphological changes in spermatogenic cells and a decrease in spermatogenic cells and a decrease in spermatogonia count (Liang et al., 2024). Nigella sativa has been shown to decrease apoptosis markers and redox status, thereby supporting our study findings (Almujaydil et al., 2023).

# **CONCLUSION**

Exposure to PS-NPs for two months did not affect body weight or organ coefficients; however, it resulted in disorders in lipid metabolism, decreased testosterone levels, and testicular tissue damage. *Nigella sativa* oil could alleviate the adverse effects of PS-NP exposure through its advantageous properties.

#### DISCLOSURE STATEMENT

The authors have no competing interests to report.

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# تأثير الجسيمات النانوية للبوليستيرين على العلاقة بين الدهون وهرمون التستوستيرون في حالات تسمم الخصية: دور زيت حبة البركة في استعادة التوازن

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كشفت هذه الدراسة عن التغيرات التي تسببها الجسيمات النانوية للبوليستيرين في مستويات الدهون و علاقتها بإفراز هرمون التستوستيرون، كما بحثت في قدرة زيت حبة البركة - وهو علاج طبيعي متاح - على إصلاح هذا الضرر. تم تقسيم عشرين فأراً ذكراً إلى أربع مجموعات بشكل عشوائي: مجموعة مرجعية لم تتلق أي علاج، ومجموعة تتوضت للجسيمات النانوية تلقت زيت حبة البركة بجرعة ٥٠٠ ملغ لكل كيلوغرام من وزن الجسم، ومجموعة رابعة تلقت كلا المادتين بنفس الجرعات. للبوليستيرين بجرعة ١٠ ملغ لكل كيلوغرام من وزن الجسم، ومجموعة رابعة تلقت كلا المادتين بنفس الجرعات تم إعطاء الجرعات عن طريق الفم ثلاث مرات أسبوعياً لمدة ستين يوماً. أظهرت النتائج أن مجموعة الجسيمات النانوية للبوليستيرين سجلت ارتفاعاً ملحوظاً في مستويات الكوليسترول والدهون الثلاثية مع الخصية تشمل تكون فجوات الدهني عالي الكثافة و هرمون التستوستيرون، بالإضافة إلى ظهور تغيرات نسيجية في الخصية تشمل تكون فجوات سيتوبلاز مية وتلف خلايا تكوين الحيوانات المنوية و از دحام الأوعية الدموية. في المقابل، أظهرت المجموعة التي تنقت الجرعات المشترك انخفاضاً في مستويات الكوليسترول والدهون الثلاثية مع الحفاظ على مستويات طبيعية من البروتين الدهني عالي الكثافة و هرمون التستوستيرون، كما لوحظت تغيرات مرضية بسيطة فقط في أنسجة من البروتين الدراسة إلى أن زيت حبة البركة يمتلك فعالية كبيرة في التخفيف من الأثار الضارة للجسيمات الخصية. تخلص الدراسة إلى أن زيت حبة البركة يمتلك فعالية كبيرة في التخفيف من الأثار الضارة للجسيمات النانوية للبوليستيرين على الهرمونات الذكرية المرتبطة باضطرابات أيض الدهون.