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Protective Role of *Nasturtium Officinale* Extract on MSG-Induced Testicular Physiological and Histological Damage in Male Albino Rats

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Abstract

Male reproductive toxicity is defined as the adverse effects of chemicals on the male reproductive system, which can lead to conditions such as infertility. This study investigates the protective potential of *Nasturtium Officinale* hydroethanolic extract (*N. Officinale*) against MSG-induced testicular toxicity in albino rats. Twenty male albino Wistar rats, ageing (8-10 weeks) and weighing (230-280 g), were randomly divided into four groups: (control, MSG (2g kg b.w. orally), MSG + 200 mg kg b.w. of *N. Officinale*, and MSG + 400 mg kg b.w. of *N. Officinale*) for six weeks. Spectroscopic analysis confirmed successful extraction of bioactive compounds, including flavonoids and phenolic acids. MSG administration caused severe testosterone suppression (0.96 ± 0.452 ng/mL) compared to the control group rats (2.539 ± 1.474 ng/mL), accompanied by dramatic downregulation of the Hsd17b3 gene and extensive histopathological damage, including seminiferous tubule degeneration and Leydig cell dysfunction. *N. Officinale* extract admiration demonstrated dose-dependent protective effects, with low-dose restoring testosterone levels (2.436 ± 0.626 ng mL) and high-dose achieving superior protection (3.624 ± 1.114 ng mL). Molecular analysis revealed significant upregulation of *Hsd17b3* in both plant extract groups compared with the MSG alone group, while histopathological examination showed remarkable tissue recovery with preserved spermatogenesis. These findings demonstrate that *Nasturtium Officinale* extract provides dose-dependent protection against MSG-induced testicular damage by supporting testosterone production, enhancing Hsd17b3 gene expression, and preserving normal testicular tissue, indicating its potential as a natural therapeutic agent for male reproductive health.

1. Introduction:

Male reproductive disorders have become increasingly prevalent across global populations, with current research demonstrating that these conditions significantly impact fertility outcomes worldwide [1], [2]. The range of these disorders includes oligospermia, asthenospermia, teratospermia, and azoospermia, which collectively represent the primary causes underlying male infertility challenges faced by partners today [3].

The development of male reproductive dysfunction involves complex disruptions affecting testicular function, sperm production regulation, and hormonal balance, nervous tissue arising from intricate interactions between genetic factors, environmental influences, and dietary components [4]. Among environmental and dietary contributors to male reproductive

problems, chemical food additives have emerged as significant concern factors, with MSG showing particularly troublesome effects on reproductive tissue health and functional capacity through documented toxic mechanisms [5].

Monosodium glutamate (MSG) stands as one of the most extensively utilized flavor enhancers in global food production, containing 78% glutamic acid,%22 sodium, and water [6]. with consumption patterns ranging from 0.3-1.0 g/day in Western populations to 4.0 g/day in Asian countries, Experimental research provides that MSG administration induced oxidative stress in testicular tissues and a decrease in the activity of the antioxidant enzyme [7]. Also has established dose-related reproductive toxicity, with MSG treatment causing significant fertility parameter deterioration even at moderate consumption levels [5], [8].

The hormonal consequences typically include decreased serum testosterone concentrations, altered luteinizing hormone secretion patterns, and compromised follicle-stimulating hormone dynamics, indicating significant disruption of the hypothalamic-pituitary-gonadal axis [9]. Histopathological examination of MSG-induced reproductive toxicity has revealed characteristic morphological changes in testicular tissues, including seminiferous tubule degeneration, Leydig cell dysfunction, and compromised Sertoli cell integrity [10].

The extensive global utilization of MSG in food processing industries and mounting evidence of its reproductive toxicity necessitate urgent development of effective protective interventions. Traditional medicine systems have historically employed medicinal plants for treating reproductive disorders [11]. Contemporary phytochemical research has validated that plant-derived bioactive compounds can effectively counteract reproductive toxicity induced by environmental and dietary factors through antioxidant mechanisms, hormonal regulation, and cellular repair processes [12], [13].

Nasturtium Officinale, commonly known as watercress, is a perennial aquatic plant belonging to the family Brassicaceae and has been traditionally utilized for its medicinal properties. Contemporary scientific research has substantiated its wide-ranging pharmacological activities, including wound-healing, antibacterial, and immunostimulant effects, largely attributed to its rich content of bioactive compounds such as flavonoids and phenolic acids [14].

Experimental studies have demonstrated that *N. Officinale* supplementation exerts pronounced protective effects on the male reproductive system in rats. It has been shown that plant extracts enhance spermatogenesis, increase sperm count and motility, and elevate serum testosterone levels, thereby mitigating chemically induced reproductive toxicity characterized by oxidative stress, hormonal disruption, and testicular tissue degeneration [11], [15]. The therapeutic efficacy of *N. Officinale* is associated with its diverse phytochemical profile, including phenolic acids, gallic acid, p-coumaric acid, quercetin, flavonoids, glucosinolates, and isothiocyanates. These constituents act synergistically to preserve normal testicular architecture by attenuating oxidative stress and preventing cellular degeneration within the seminiferous tubules [16], [17].

Histopathological investigations have revealed improved germ cell organization and preservation of Leydig and Sertoli cell integrity following *N. Officinale* administration, underscoring its restorative and cytoprotective roles in testicular function. Furthermore, controlled experimental models consistently report that *N. Officinale* supplementation effectively ameliorates reproductive impairment induced by diverse environmental toxicants and pharmacological agents, reflected by improved testicular histology, normalized hormonal profiles, and enhanced sperm parameters [18], [19].

Hydroxysteroid 17-beta dehydrogenase type 3 (HSD17B3) is a microsomal NADPH-dependent oxidoreductase enzyme that plays a critical role in testicular steroidogenesis by catalyzing the irreversible conversion of androstenedione to testosterone—the final and rate-limiting step in androgen biosynthesis [20]. Encoded by the Hsd17b3 gene, it belongs to the short-chain dehydrogenase/reductase (SDR) superfamily and exhibits high tissue specificity, with predominant expression in Leydig cells of the adult testis [21]. HSD17B3 is essential for establishing and maintaining normal testosterone levels, supporting male reproductive function, spermatogenesis, and secondary sex characteristics. Its expression is highly sensitive to environmental and chemical insults, including oxidative stress, heavy metals, and endocrine disruptors, which can impair both its transcription and enzymatic activity [22].

In experimental models, administration of monosodium glutamate (MSG) has been shown to suppress Hsd17b3 expression, contributing to disrupted testosterone synthesis and testicular dysfunction [23]. Supplementation with *N. Officinale* has been shown to effectively ameliorate reproductive damage induced by various environmental toxicants and pharmaceutical agents. The plant extract exhibits strong antioxidant and anti-inflammatory properties, leading to improved sperm concentration, motility, and hormonal balance. In the context of MSG induced toxicity, *N. Officinale* may support the recovery of testicular function by restoring Hsd17b3 expression levels and normalizing steroidogenic pathways [24].

This study aimed to investigate the protective effects of *Nasturtium Officinale* hydroethanolic extract against monosodium glutamate-induced testicular toxicity in male albino rats. The research sought to evaluate dose-dependent therapeutic efficacy through comprehensive assessment of serum testosterone concentrations, Hsd17b3 gene expression patterns in blood and testicular tissues, and histopathological alterations in testicular architecture. Additionally, the study aimed to characterize the bioactive phytochemical composition of the extract using UV-Vis and FTIR spectroscopic analyses to establish the mechanistic basis for its protective effects on male reproductive function.

2. Material and Methods:

2.1 Plant Material Collection and Extract Preparation:

2.1.1 Plant Collection:

Fresh aerial parts of *N. Officinale* (watercress) were collected from Barzewa village, Erbil Governorate, Iraq, during the optimal growth season (May to July). The plant specimens were botanically identified and authenticated by Prof. Dr. Abdulla Shukr Sardar, Professor of Biology Department, College of Education, Salahaddin University, Erbil. A voucher specimen was deposited in the herbarium for future reference and taxonomic verification.

2.1.2 Extract Preparation:

The collected leaves underwent thorough surface cleaning with distilled water (three successive washes) to eliminate dirt and contaminants. The cleaned plant material was air-dried in shade at room temperature (22-25^{o}C) for ten days to preserve thermolabile compounds. The dried leaves were mechanically ground using a mortar and pestle to obtain fine powder [13], [16].

Hydroethanolic extraction was performed using the maceration method. Twenty-five grams of powdered *N. Officinale* leaves were macerated in 500 ml of hydroethanolic solvent (70% ethanol:30% distilled water, v/v) and heated at 60^{o}C for 30 minutes under continuous stirring. The mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. After 4 days of maceration at 37^{o}C, the extract underwent secondary filtration and concentration under reduced pressure using a rotary evaporator [11]. The concentrated extract was transferred to sterile Petri dishes and dried in an oven at 40^{o}C until complete solvent evaporation. The final dried extract was stored at -20^{o}C until experimental use, ensuring stability and preventing degradation [16].

2.1.3 Animal Housing and Experimental Design:

Twenty healthy adult male Albino rats (Rattus norvegicus) aged 8-10 weeks (230-280g body weight) were procured from the animal house facility (Animal Breeding Center, Jihan University, Erbil) after comprehensive health screening. Animals were maintained in standard laboratory cages under controlled environmental conditions: temperature 22±2^{o}C, relative humidity 50-60%, and a 12-hour light/dark cycle, with standard laboratory chow and tap water ad libitum. After a 7-day acclimatization period preceded experimental procedures to minimize stress-related variables.

2.1.4 Experimental Design and Treatment Protocol:

Following comprehensive body weight assessment, animals were systematically randomized into four equal groups (n=5 per group) using a completely randomized design (CRD) with daily oral gavage administration for 6 consecutive weeks: Group 1 (Control) received standard laboratory chow and tap water ad libitum without pharmacological intervention; Group 2 (MSG-treated) received monosodium glutamate (MSG) at 2g Kg body weight daily to induce testicular toxicity; Group 3 (MSG + Low-dose extract) received MSG (2g kg) followed by *N. Officinale* hydroethanolic extract at 200mg kg body weight one hour later; and Group 4 (MSG + High-dose extract) received MSG (2g kg) followed by *N. Officinale* hydroethanolic extract at 400mg kg body weight one hour later. The staggered administration protocol was designed to prevent potential chemical interactions while ensuring optimal bioavailability of protective compounds.

2.2 Sample Collection and Processing:

2.2.1 Animal Dissection and Blood Collection:

At the experimental endpoint (week 6), animals were fasted for 12 hours with free access to water. Anesthesia was induced using intramuscular injection of xylazine-ketamine mixture (1:9 ratio) as a single dose. Blood samples were collected via cardiac puncture using sterile disposable syringes: 2ml in EDTA-containing tubes for hematological analysis and 5ml in gel tubes for biochemical assays. Serum was obtained by allowing blood to clot at room temperature for 15 minutes, followed by centrifugation at 3000 rpm for 15 minutes. Serum samples were stored at -20^{o}C until biochemical and antioxidant analyses [8].

2.2.2 Organ Collection and Processing:

Testicular tissues were carefully dissected, washed in cold phosphate-buffered saline (PBS), and blotted with filter paper. Fresh organ weights were recorded using a Shimadzu digital analytical balance (sensitivity 0.001g). Tissues were divided for multiple analyses: sections were preserved in PBS and stored at -80^{o}C for molecular studies (Hsd17b3 gene expression analysis), while the remaining tissues were fixed in 10% neutral buffered formalin for histopathological examination [25].

2.3 The Characterization of Synthesized *N. Officinale* hydroethanolic Extract:

2.3.1 Visible-UV Spectroscopy:

An ultraviolet-visible spectrum investigation verified the formation of N. Officinale hydroethanolic extract. The absorbance spectrum was obtained utilizing UV-visible spectroscopy (Perkin Elmer Spectrophotometer) at a wavelength between 200 and 700 nm.

2.3.2 FTIR Measurement:

The functional groups of produced N. Officinale hydroethanolic extract were investigated utilizing an FT-IR instrument (Shimadzu Company, Kyoto, Japan) to identify distinctive bands spanning between 400 and 4000 cm-1 with an intention of 2 cm^{-1}.

2.3.3 Determination of Testosterone Level:

The collected serum samples were analyzed for testosterone levels using the Cobas e 411 analyzer (Roche Diagnostics,

Mannheim, Germany), an automated electrochemiluminescence immunoassay (ECLIA) system. Serum samples (20 μL) were processed according to the manufacturer′s protocols, and results are expressed in ng mL.

2.4 Molecular Analysis:

2.4.1 RNA extraction protocol:

Total RNA was extracted utilizing the AddPrep Total RNA Extraction Kit (AddBio Com, Korea) in accordance with the manufacturer′s guidelines.

2.4.2 Quantitative RT-PCR for expression detection of mRNAs:

Total RNA was extracted utilizing the Total RNA Extraction Kit (AddBio, Korea) following the manufacturer's guidelines. The RNA quality was assessed with a Nano Drop spectrophotometer (Biometrics, Wilmington, USA) by determining the absorbance ratio at 260/280 nm. The synthesis of cDNA of mRNAs was analyzed by performing the cDNA Synthesis Kit (AddBio Com, Korea), following the manufacturer's instructions, performing the thermal cycling with priming at 25^{o}C for 10 min, reverse transcriptase at 50^{o}C for 60 min, RT inactivation at 80^{o}C for 5 min, and finally holding at 12^{o}C. Unique RT primers were utilized to evaluate the expression levels of mRNAs, as revealed in (Table 1). The qRT-PCR experiments were performed to evaluate mRNA expression by applying the SYBR green method, subsequent the manufacturer's guidelines (AddScript 2×SYBR Master) (AddBio, Korea) and qRT-PCR CFX96 (Bio-Rad, USA). A specific forward and universal reverse primer for Hsd17b3 was used in the qRT-PCR cocktail. The primer sequences applied in this research were incorporated into each PCR tube, with 20 μL volume of for the RT-PCR process, the cocktail was: 5 μL of cDNA template; 3 μL of nuclease-free water; 1.0 μL of primers with 10 pmol/μL; and 10 μL of SYBR Green Master Mix (without ROX). The PCR programs for Hsd17b3 and the internal controls GAPDH was 95^{o}C for 3 min, following 40 cycles of denaturation at 95^{o}C for 25 sec, annealing (56.5^{o}C for hsd17b3, and 54^{o}C for GAPDH) for 60 sec, extension at 72^{o}C for 45 sec, and the final extension step was performed one cycle at 72^{o}C for 5 min. The CT values for the samples were normalized to GAPDH expression as an internal reference, and the data were then converted into relative fold change using the $2^{\Delta\Delta CT}$ algorithm.

2.5 Histopathological Examination:

Fixed testicular tissues underwent standard histological processing, including 24-hour formalin fixation, dehydration through graded alcohols (70%, 95%, 100%), clearing in xylene, and paraffin embedding. Tissue sections (6μm thickness) were cut using a rotary microtome, deparaffinized, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope (BH Olympus, Japan) for morphological changes and photographically documented [17].

2.6 Statistical Analysis:

Statistical analysis was performed using GraphPad Prism software (version 9.01, USA). Data normality was assessed using the Shapiro-Wilk test, followed by ONE-WAY ANOVA and Tukey′s post-hoc test for multiple group comparisons. Results are expressed as mean ± standard deviation (Mean ± SEM) with statistical significance set at p-value $\leq$ 0.05. Different alphabetical superscript letters indicate significant differences between groups, while identical letters denote no significant differences.

3. Results:

3.1 UV-Vis Spectrophotometric Assessment:

UV-visible spectroscopy using a (Perkin Elmer Spectrophotometer) showed that the hydroethanolic extract of *N. Officinale* displayed characteristic phenolic compound absorption patterns, with a primary maximum at 318 nm (A = 1.190) indicating flavonoid presence and secondary bands at 230-280 nm (A = 0.735 at 230 nm) confirming hydroxycinnamic acid derivatives (Figure 1). The absorption decreased beyond 350 nm to baseline values, demonstrating successful polar phenolic extraction without chlorophyll interference. These results align with the established phytochemical profile of *N. Officinale* and confirm the efficacy of hydroethanolic extraction for bioactive metabolite recovery.

3.2 FTIR Spectroscopic Analysis:

The functional groups of the *N. Officinale* hydroethanolic extract were analyzed using FT-IR spectroscopy (Shimadzu, Kyoto, Japan) in the range of 400-4000 cm^{-1} with 2 cm^{-1} resolution. The spectrum showed (Figure 2) characteristic peaks at 3323.35 cm^{-1} (O-H stretching of phenolic compounds), 2924.09 and 2852.72 cm^{-1} (C-H stretching of aliphatic compounds), 1737.86 cm^{-1} (C=O stretching of esters/organic acids), and 1604.77 cm^{-1} (C = C aromatic stretching of flavonoids). Additional bands in the fingerprint region (1369.46, 1230.58, 1051.20, and 991.41 cm^{-1}) confirmed the presence of polyphenolic structures typical of plant extracts.

3.3 Serum Testosterone Concentrations:

Following six weeks of daily oral gavage administration, serum testosterone concentrations showed marked differences between experimental groups (Figure 3). The control group (N) maintained normal testosterone levels at 2.539 ± 0.6593 ng mL. Daily MSG treatment (P) at 2g kg body weight resulted in significant testosterone suppression to 0.960 ± 0.2023ng mL. Treatment groups receiving N. Officinale hydroethanolic extract one hour after MSG administration demonstrated protective effects. Group G3 (MSG + 200mg kg extract) showed restored testosterone levels into the normal range (2.436 ± 0.2801 ng mL), while Group G4 (MSG + 400mg kg extract) exhibited elevated testosterone concentrations into 3.624 ± 0.4983ng mL (Table 1). Statistical evalu-

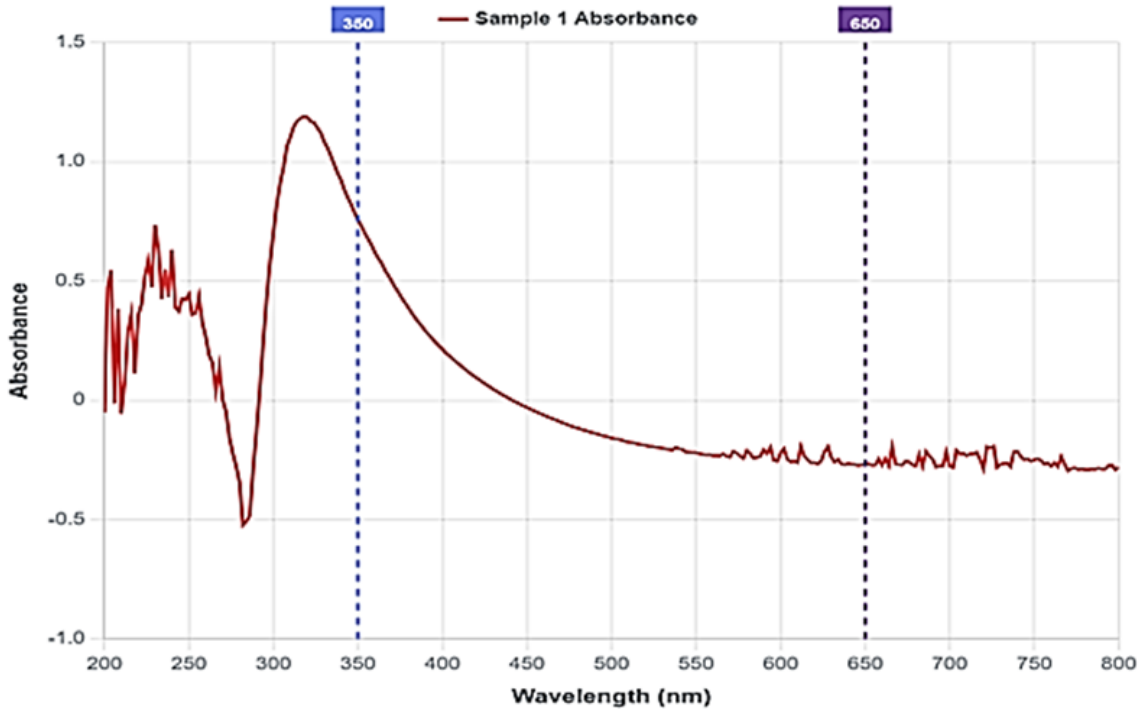

Figure 1. UV-VIS absorption spectrum of hydroethanolic extract of *N. Officinale*.

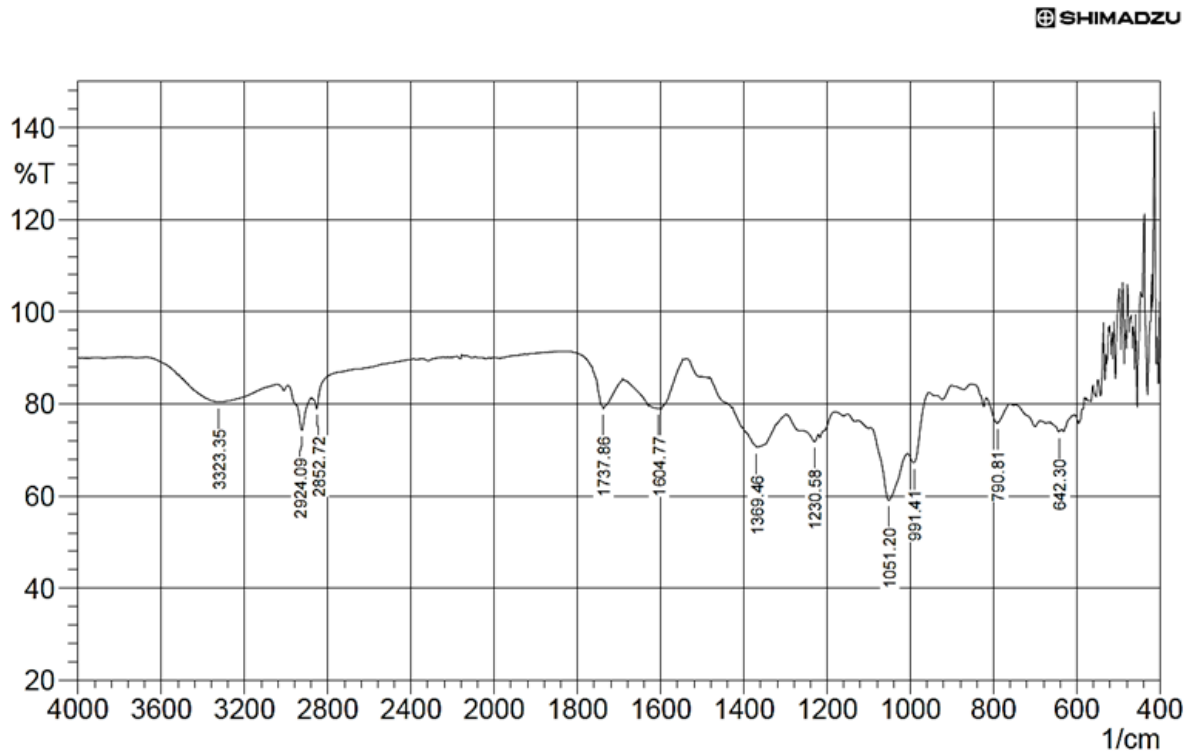

Figure 2. Fourier-transform infrared (FTIR) spectrum of *N. Officinale* hydroethanolic extract displaying characteristic vibrational frequencies of bioactive functional groups (4000-400 cm^{-1}).

Table 1. Oligonucleotide primers used for qRT-PCR for mRNA expression.

Gene name	Primer	Sequence References
Hsd17b3-F	5-ATT ACC TCC GTA GTC AAG A -3	
Hsd17b3-R	5- TAT TCC ACA TTC AAA GCC T-3	
GAPDH	Forward 5- AAG AAG GTG GTG AAG CAG GCATC-3	[25]
GAPDH	Reverse 5CGA AGG TGG AAG AGT GGG AGTTG-3	

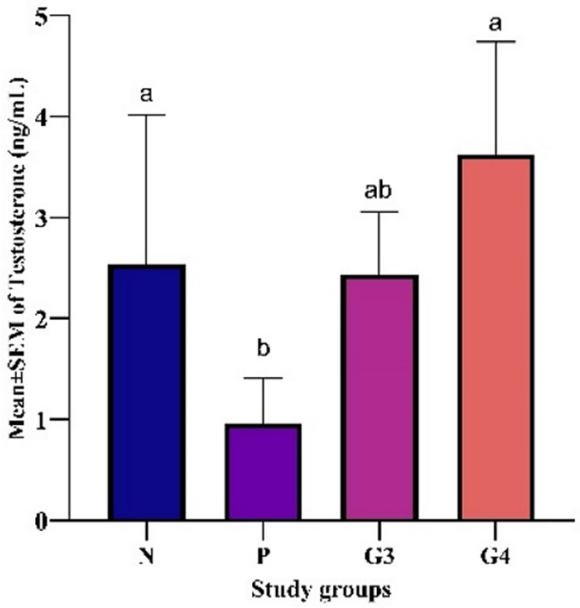

Figure 3. Serum testosterone concentrations in experimental groups showing protective effects of *N. Officinale* extract against MSG-induced suppression.

ation using one-way ANOVA followed by Tukey′s multiple comparison test indicated significant treatment effects. The control (N), low-dose extract (G3), and high-dose extract (G4) groups demonstrated Statistical uniformity (p-value=0.9984 and p-value=0.3497, respectively), sharing the same alphabetical designation a′. Conversely, the MSG-treated group (P) exhibited statistically distinct values, denoted by letter ′b′, indicating significant deviation from all other experimental conditions.

3.4 Expression of the Hsd17b3 gene:

The qRT-PCR data in blood samples indicated a substantial downregulation in the average levels of Hsd17b3 expression in positive control (0.03528±0.0096) when compared to negative controls (negative control 0.9992±0.0908) but not reached significant levels of expression (p-value=0.025). Conversely, the expression level of Hsd17b3 in both high dose and low dose of alcoholic extract groups was upregulations and significantly different from that of positive controls (3.381±0.4329, p-value<0.0001, and 1.197±0.06935, p-value=0.0262, respectively), as illustrated in Figure (4 A).

Similarly, the expression levels of Hsd17b3 gene in testicular tissue (Figure 4 B) samples was considerably reduced in positive groups compared to negative controls (negative controls(G1): 0.9988±0.3230; positive controls(G2): 0.004128 ± 0.00145; p-value = 0.0133). Conversely, the expression levels of Hsd17b3 gene in both low dose(G3) and high dose(G4) of plant extracts was significantly upregulated compared to samples from positive control, returning to levels observed in negative controls (1.328±0.03795, p-value=0.0024, and 1.409±0.08933, p-value=0.0016 respectively). As mentioned earlier, the expression data were standardized to the internal control (GAPDH) before analysis. Data are presented as mean values with corresponding standard errors (SEM) (Table 2).

3.5 Histological Assessment:

The histological study showed various alterations in testicular tissue of treated groups in comparison to the control group, which showed normal histological patterns with well-organized seminiferous tubules, intact basement membranes, and complete spermatogenic cycles (Figure 5: A and B). MSG administration caused severe testicular damage with seminiferous tubular degeneration, decrease in the germ cells population, seminiferous tubules surrounded by edematous stroma containing small groups of Leydig cells, dilated blood vessels (Figure 5: C), seminiferous tubules filled by spermatogenic cells up to spermatid only with no sperm formation or defect sperm formation (spermatogenic arrest), (Figure 5: D). Animals receiving 200 mg kg (b.w.) of *N. Officinale* ethanoic extract after MSG administration demonstrated partial structural recovery with enhanced cellular organization, reduced vacuolization, and increased mature spermatozoa (Figure 6: A and B). While testicular tissue in the group received 400 mg kg (b.w) of the plant extract treatment provided exceptional protection with normal tubular integrity, complete spermatogenic restoration, abundant mature spermatozoa, and healthy interstitial tissues (Figure 6: C and D).

4. Discussion:

This comprehensive investigation demonstrates the multifaceted protective effects of *N. Officinale* hydroethanolic

Table 2. Serum testosterone concentrations in experimental groups.

	Study groups			
	NC	PC	G3	G4
Testosterone (ng mL)	2.539 ±0.659a	0.960 ±0.2023b	2.436 ± 0.2801ab	3.624±0.4983a

*: Values represent mean ± SEM (ng mL). NC: normal control; PC: MSG (2 g Kg b.w.); G3: MSG + *N. Officinale* (200 mg kg b.w.); G4: MSG + *N. Officinale* (400 mg kg b.w.). b.w. = body weight.

Table 3. Relative expression ($2^{\Delta\Delta CT}$) of different Hsd17b3 gene among study groups.

Sample types	Study groups			
	NC	PC	G3	G4
Blood	0.9992±0.09083b	0.03528±0.009689c	1.197±0.06935b	3.381±0.4329a
Tissue	0.9988±0.3230b	0.004128±0.00145d	1.328±0.03795b	1.409±0.08933a

*: Fold change is obtainable as Mean±SEM. *p-value ≤0.05 is measured as significant different between study groups. The total expression displays changes in expression between the treated groups and the negative groups.

extract against MSG-induced testicular toxicity through integrated hormonal, molecular, and histopathological analyses that collectively establish the plant extract's exceptional therapeutic potential for treating environmentally induced male reproductive dysfunction.

The spectroscopic characterization using UV-Vis and FTIR techniques confirmed successful extraction of bioactive constituents, with UV-Vis analysis revealing characteristic polyphenolic absorption patterns at 318 nm (A = 1.190), confirming flavonoid compounds, particularly quercetin and kaempferol derivatives, and this is consistent with a previous report [26]. This wavelength is aligned with the standard uptake properties. Prior theoretical research indicated that this flavonoid presents unique UV-Vis spectral properties in the 300-320 nm range due to its conjugated π-electron system, and its well-documented antioxidant properties and therapeutic potential [27].

Additional absorption bands observed at 230–280 nm validated the presence of hydroxycinnamic acid derivatives, including caffeic and chlorogenic acid compounds, which are typical of phenolic substances due to their aromatic structure and extended conjugation patterns, in agreement with previous studies [28], [29] These compounds are commonly found throughout Brassicaceae plants and demonstrate powerful antioxidant and anti-inflammatory properties, with caffeic and chlorogenic acid derivatives exhibiting protective effects against oxidative damage [30], [31].

In the present study, the FTIR analysis helped us identify the molecular structure, which shows a broad absorption peak at (3323.35 cm^{-1}), so this peak indicates (O-H) stretching vibration from the phenolic hydroxyl group, the key component that gives the compound its antioxidant properties [32]. This unique absorption band (3200–3400 cm^{-1}) demonstrates O-H, hydrogen-bonded stretching vibrations, characteristic of polyphenolic compounds, while C-H stretching vibrations (2924.09 and 2852.72 cm^{-1}) confirmed glycosidic linkages associated with flavonoid glycosides; the same results were consistent with prior studies [33]. The absorptions in the fingerprint region represent molecular evidence for the structural aspects of polyphenolic compounds and glucosinolates typical of Brassicaceae plant chemistry [34]. Notably, the peak at 2924 cm^{-1} shows significant overlapping with methylene scissoring vibrations and may also be related to methoxy compounds, while the peak at 2852 cm^{-1} corresponds specifically to C-H symmetric stretching vibrations, these were supported by previous observations [35].

The hormonal analysis showed significant dose-dependent protective effects against MSG-induced testosterone reduction, offering strong evidence for the extract's ability to restore hormonal balance, aligning with previous findings [25], [36]. MSG treatment at 2g/kg body weight for six weeks caused severe testosterone reduction to 0.960 ± 0.202 ng mL ($P < 0.001$), confirming the established mechanism of MSG-induced reproductive damage through disruption of the hypothalamic-pituitary-gonadal (HPG) axis, also confirmed by the studies of [5], [37].

Recent comprehensive reviews have found that MSG treatment lowers testosterone levels, gonadotropin-releasing hormone, and luteinizing hormone through neurotoxic effects that cause brain cell damage via disruption of the hypothalamic-pituitary axis pathway. Consistent with earlier reports, [8], [9]. Our findings demonstrate that treatment with a low-dose extract of $N. Officinale$ (200mg kg) shows significant therapeutic potential by fully reverting MSG-induced reductions in testosterone levels to serum concentrations of 2.436 ± 0.2801 ng mL and fully restoring to control values ($P > 0.05$ vs. control) , this biological restoration approach is based on the composition of the extracts and their rich supply of plant compounds that work together through high concentration of secondary metabolites and especially phenolic compounds to

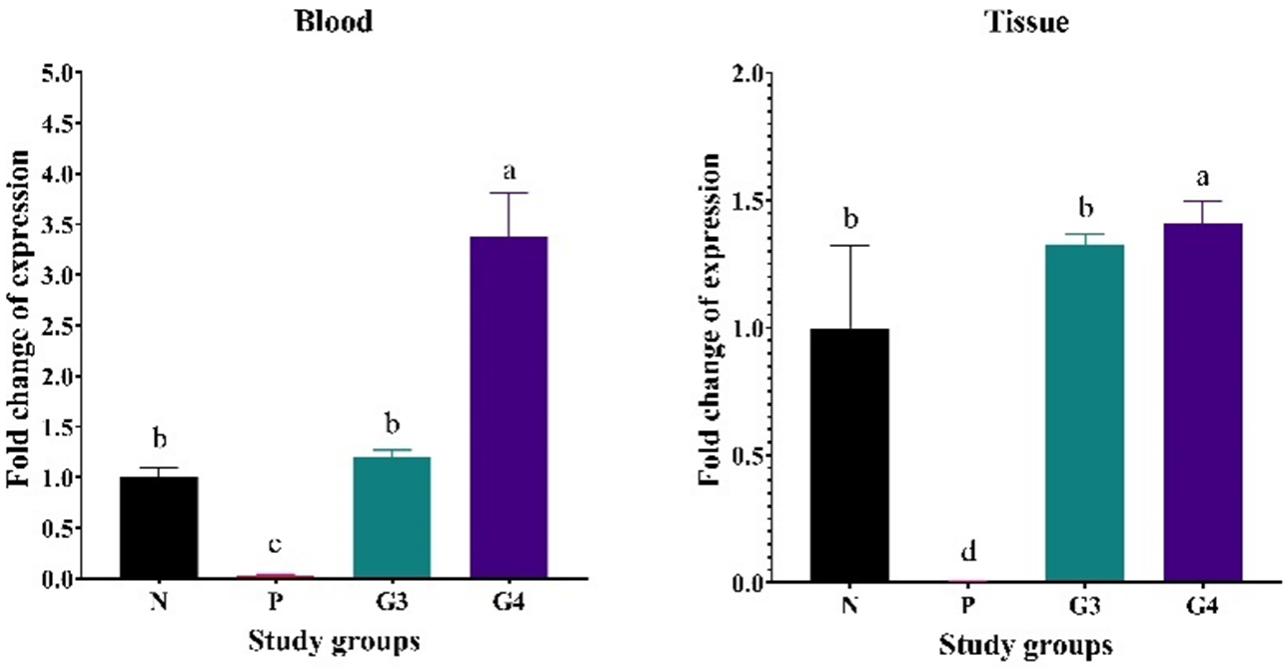

Figure 4. Expression fold change ($2^{\Delta\Delta CT}$) of the Hsd17b3 gene in treated groups compared to both positive and negative controls. (A). Blood samples, and (B). Testis tissue samples.

reduce neuroendocrine damage, supported by previous studies [13], [16]). The analysis of a wide range of plant compounds found in watercress extracts demonstrated compelling strong antioxidant chemicals, including rutin, coumaric acid, ferulic acid, L-serine, L-proline, and phytol, and showed protective influences to protect against toxicity [11], [38].

High-dose extract treatment (400mg kg) presented a higher medicinal effect, raising testosterone to 3.624 ± 0.4983ng mL, surpassing both negative control and MSG-treated groups, in agreement with earlier studies [15], [19].This improvement beyond normal physiological levels suggests that N. Officinale bioactive compounds not only protect against damage but also actively stimulate steroidogenic pathways through enhanced Leydig cell function and enhanced testosterone synthesis [17], [39].

The molecular analysis provided crucial mechanistic insights through examination of Hsd17b3 gene expression patterns, revealing the direct relationship between gene expression, enzyme activity, and testosterone biosynthesis [40]. MSG administration caused dramatic downregulation of Hsd17b3 expression (0.035 ± 0.009 in blood; 0.004 ± 0.001 in tissue) (Table 2), directly correlating with the observed testosterone suppression and confirming the critical role of this enzyme in male reproductive function, consistent with prior reports [41].

Hsd17b3 (17β-hydroxysteroid dehydrogenase type 3) represents the rate-limiting enzyme in the final step of testosterone biosynthesis, catalyzing the conversion of androstenedione to testosterone in Leydig cells [42]. Approximately 95% of circulating testosterone is synthesized by the testis, with the final step in this canonical pathway controlled by the activity of Hsd17b3. Recent research suggests that Hsd17b3 acts as a rate-limiting step in testosterone production, and in its absence, the hypothalamus-pituitary-gonadal axis responds in a manner consistent with compensated Leydig cell failure [22].

The profound suppression of Hsd17b3 expression following MSG administration demonstrates how environmental toxicants can disrupt steroidogenic enzyme expression at the transcriptional level [43]. Both low-dose and high-dose N. Officinale extract treatments successfully upregulated Hsd17b3 expression, with high-dose treatment showing particularly robust gene activation (3.381 ± 0.432 in blood; 1.409 ± 0.089 in tissue). This molecular restoration provides direct evidence that the plant extract protects testosterone synthesis by preserving key steroidogenic enzyme expression through multiple protective mechanisms, echoing previous findings [44], [45]. The mechanism underlying *N. Officinale*-mediated gene expression restoration likely involves the plant's powerful antioxidant compounds neutralizing reactive oxygen species that would otherwise damage cellular DNA and disrupt transcriptional machinery, and this underpin by other studies [16], [17].

The histopathological examination provided comprehensive morphological evidence demonstrating the dose-dependent

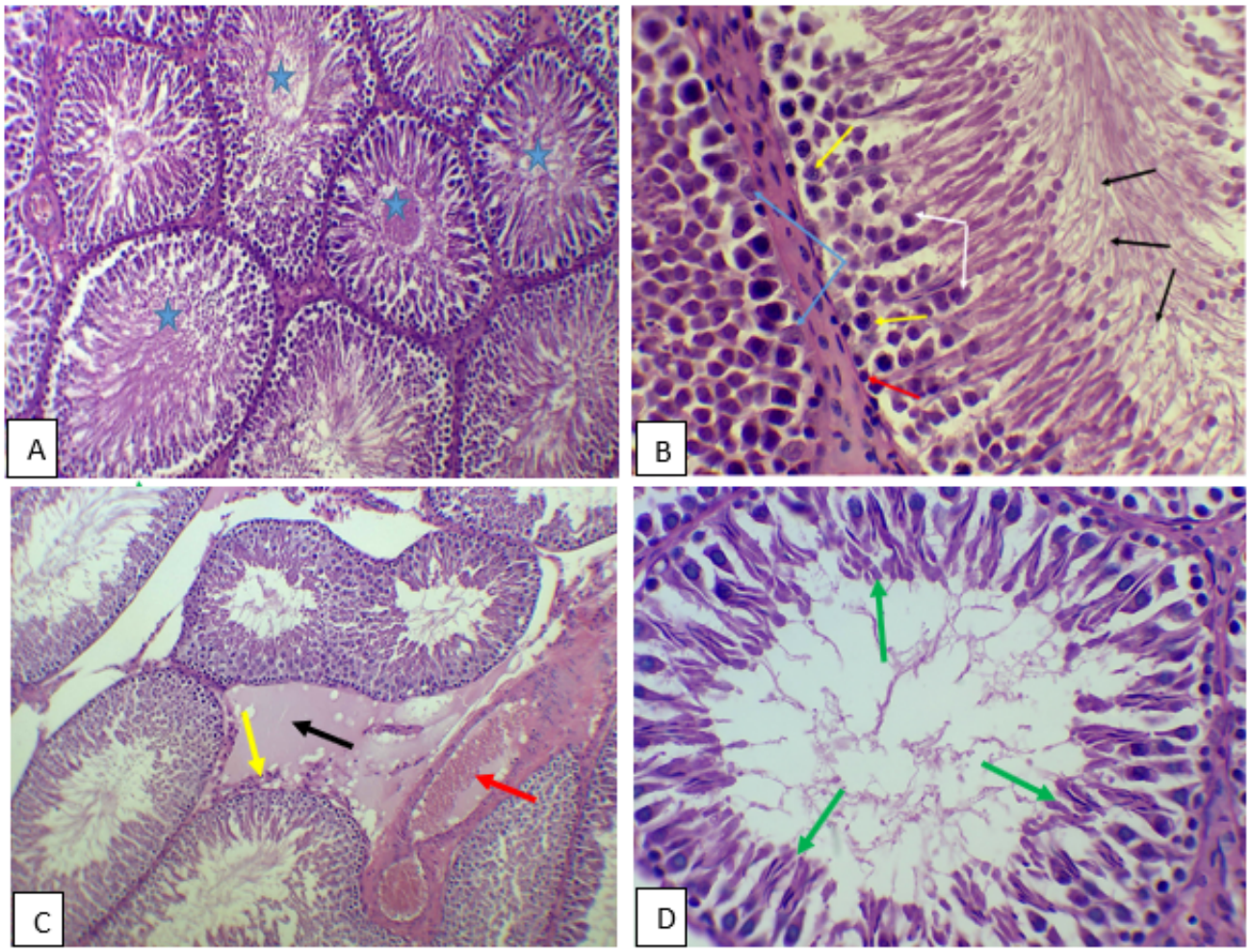

Figure 5. :A & B): Histological sections through the testes of control group rats showing, normal feature of seminiferous tubules (★), sertoli cells (blue arrow), spermatogonia (red arrow), primary spermatocyte (yellow arrow), spermatids (white arrow) and spermatozoa (black arrow), C, D) MSG treated group rats showing, edematous stroma (black arrow), dilated blood vessels (red arrow), leydig cells (yellow arrow); spermatogenic arrest (green arrow); H&E; A&C (100x), B&D (400x).

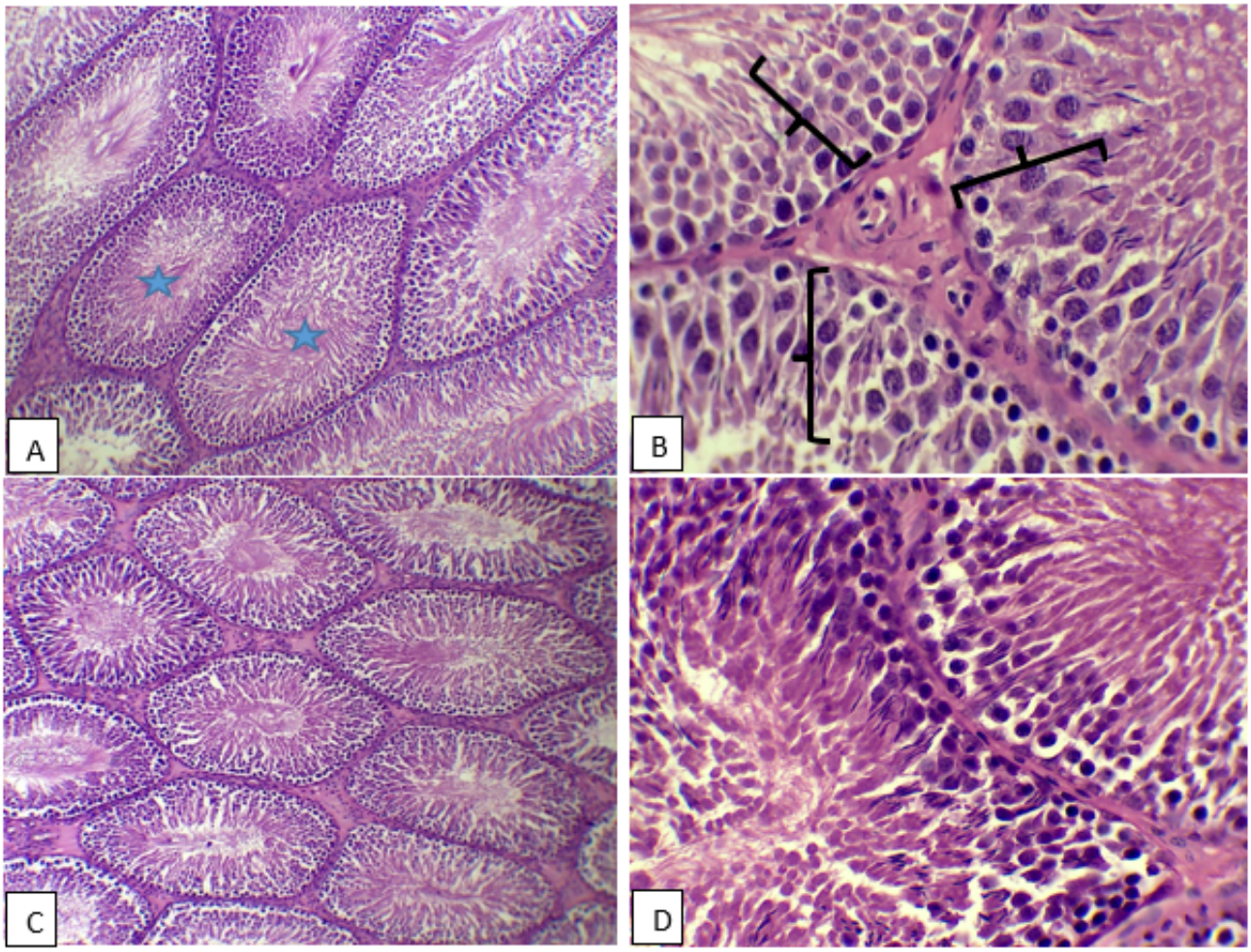

Figure 6. Histological section through the testes of (MSG + 200 mg kg b.w. of *N. Officinale*) treated group rats showing, A) normal seminiferous tubules with high spermatogenic cells at different levels (★), B) normal spermatogenic layer (black brackets), C&D testes of (MSG + 400 mg/kg b.w. of *N. Officinale*) treated group rats showing approximately normal seminiferous tubules full of normal sperm and spermatogenic layer, H&E; A&C (100x), B&D (400x).

protective effects of *N. Officinale* extract against MSG-induced testicular toxicity through systematic evaluation of seminiferous tubule architecture, spermatogenic cell populations, and interstitial tissue integrity [46]. The dose-dependent histological recovery observed with N. Officinale treatment reflects the extract's multifaceted protective mechanisms operating at the cellular, molecular, and systemic levels. These observations were in agreement with previous studies [16]. Watercress extracts contain strong antioxidant and antimutagenic substances, including rutin, coumaric acid, ferulic acid, L-serine, L-proline, and phytol, which contribute to protective effects against cellular toxicity [47], [48].

The histological analysis indicates that *N. Officinale* extract mitigates MSG- induced alteration, Sach as cellular vacuolization, nuclear abnormalities, and spermatogenic disruption, thereby maintaining the normal structure and organization of seminiferous [13]. Furthermore, the combined evaluation of hormonal, molecular, and histopathological data suggests *N. Officinale* exserts it's protective effect through many complementary mechanisms, offering comprehensive protection [12], [49].

Our results revealed that restoration of testosterone levels correlates directly with Hsd17b3 gene upregulation, which in turn supports the histological recovery observed in testicular tissues. This multi-level protection demonstrates the ability of *N. Officinale* extract's exceptional therapeutic potential for treating environmentally-induced reproductive dysfunction, particularly given the widespread MSG administration and dietary intake of MSG and other environmental toxicants in modern diets, so that confirming previous observations [5], [37].

5. Conclusion:

The present study findings indicate that the hydroethanolic extract of N. Officinale (low- and high-dose) can significantly protect against monosodium glutamate (MSG)-induced testicular injury in albino rats. The extract was able to completely reverse the MSG-induced reproductive intoxication by acting on testosterone levels, preserving steroidogenic enzyme expression, and maintaining normal testicular histoarchitecture. The high-dose treatment for N. Officinale outperformed the low-dose treatment, partially increasing parameters above normal levels, while preventing seminiferous tubule degeneration and Leydig cell dysfunction. Collectively, the results documented above demonstrate the plant extract's remarkable therapeutic potential for treatment of environmental health-induced male reproductive dysfunction and should be advanced into clinical investigation.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The animal experimentation protocol was approved by the Institutional Animal Ethics Committee of Salahaddin University College of Education.

Author Contributions: Hemin M. Rahman conducted the experimental work, data collection and analysis, and wrote the manuscript as part of her master's degree research. Dr. Treefa F. Ismael designed the study methodology, conceptualized the work, and provided supervision and editorial review. Both authors approved the final manuscript and agree to be accountable for all aspects of the work.

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الدور الوقائي لمستخلص نبات الجرجير (*Nasturium Officinale*) ضد التلف الفسيولوجي والنسيجي في الخصيتين الناجم عن غلوتامات احادي الصوديوم (*MSG*) في ذكور الجرذان البيضاء

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الخلاصة

تُعرَفْ السمية الإنجابية الذكورية بأنها التأثيرات الضارة للمواد الكيميائية على الجهاز التناسلي الذكري، والتي يمكن أن تؤدي إلى حالات مرضية مثل العقم. تبحث هذه الدراسة في الإمكانات الوقائية للمستخلص الهيدروإيثانولي لنبات الجرجير (*Nasturium Officinale*) ضد السمية الخصوية المحدثة بواسطة غلوتامات أحادي الصوديوم في الجرذان البيضاء. تم تقسيم عشرين من ذكور جرذان الويستار البيضاء، التي تتراوح أعمارها بين (10-8 أسابيع) وأوزانها بين (230 ـ 280 غرام)، عشوائياً إلى أربع مجموعات: (المجموعة الضابطة، مجموعة غلوتامات أحادي الصوديوم بجرعة 2 غرام من وزن الجسم عن طريق الفم، مجموعة غلوتامات أحادي الصوديوم + 200 ملغم من وزن الجسم من مستخلص الجرجير، ومجموعة غلوتامات أحادي الصوديوم + 400 ملغم من وزن الجسم من مستخلص الجرجير) لمدة ستة أسابيع. أكد التحليل الطيفي نجاح استخلاص المركبات النشطة حيوياً بما في ذلك الفلافونويدات والأحماض الفينولية. تسبب إعطاء غلوتامات أحادي الصوديوم في انخفاض شديد لمستوى هرمون التستوستيرون (0.96 ± 0.452 نانوغرام مقارنةً بجرذان المجموعة الضابطة (2.539 ± 1.474 نانوغرام مصحوباً بانخفاض كبير في التعبير الجيني لجين *Hsd17b3* وحدوث أضرار نسيجية مرضية واسعة النطاق تشمل تنكس الأنابيب المنوية وخلل وظيفي في خلايا لايديغ. أظهر إعطاء مستخلص الجرجير تأثيرات وقائية معتمدة على الجرعة، حيث أدت الجرعة المنخفضة إلى استعادة مستويات هرمون التستوستيرون (2.436 ± 0.626 نانوغرام بينما حققت الجرعة المرتفعة حماية متفوقة (3.624 ± 1.114 نانوغرام كشف التحليل الجزيئي عن زيادة معنوية في التعبير الجيني لجين *Hsd17b3* في كلتا مجموعتي المستخلص النباتي عند مقارنتهما بمجموعة غلوتامات أحادي الصوديوم وحدها، في حين أظهر الفحص النسيجي المرضي تعافياً ملحوظاً في الأنسجة مع الحفاظ على عملية تكوين الحيوانات المنوية. توضح هذه النتائج أن مستخلص الجرجير يوفر حماية معتمدة على الجرعة ضد الأضرار الخصوية المحدثة بواسطة غلوتامات أحادي الصوديوم من خلال دعم إنتاج هرمون التستوستيرون، وتعزيز التعبير الجيني لجين *Hsd17b3* ، والحفاظ على الأنسجة الخصوية الطبيعية، مما يشير إلى إمكانياته كعامل علاجي طبيعي لصحة الإنجاب الذكورية.

الكلمات الدالة : السمية الإنجابية، غلو تامات أحادي الصوديوم، التستوستيرون، الجرجير (*Nasturium Officinale*) ،جين *Hsd17b3* .

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: تمت الموافقة على بروتوكول تجارب الحيوانات من قبل لجنة أخلاقيات الحيوان المؤسسية في كلية التربية، جامعة صلاح الدين.

مساهمات المؤلفين: أجرى همن مولود رحمن العمل التجريبي، وجمع البيانات وحلّله، وكتب المخطوطة كجزء من بحث رسالة الماجستير الخاصة به، قامت الدكتورة تريفة فاروق إسماعيل بتصميم منهجية الدراسة، ووضع الإطار المفاهيمي للعمل، وقدمت الإشراف والمراجعة التحريرية، وافق كلا المؤلفين على النسخة النهائية من المخطوطة ويتحملان المسؤولية الكاملة عن جميع جوانب هذا العمل.