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Original Article

Therapeutic Potential of *Withania somnifera* on Nandrolone Decanoate Induced Gonadal Toxicity

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ABSTRACT

Background: Nandrolone decanoate (ND) is a commonly used androgen-based steroid that causes adverse effects on illicit self-administered users to boost their physical endurance. Although, it has an anabolic effect on the skeletal muscles, plausible evidence supports that the gonadal toxicity of ND can cause damage to reproductive functions. **Aim:** The present study investigated the possible protective effects of *Withania somnifera* (WS) root extract (200 and 400 mg/kg bw) on ND-induced (10 mg/kg bw) male gonadal toxicity in rats. **Materials and Methods:** The present seminal research was not experimented in any of the earlier studies. ND was induced in rats by intramuscular injection bi-weekly for 30 days. The aqueous extract of WS root powder was delivered orally once daily for 30 days to the ND-treated group. Hematoxylin-and-eosin-marked sections of the testes were studied. **Results:** The testosterone levels significantly decreased while there was an increase in the malondialdehyde levels among the ND-induced group against the control group. The ND induced rats treated with varying doses of WS showed a significant reversal of the gonadal toxicity by improvement in the testosterone and antioxidant parameters. The sperm count and sperm motility rate were increased significantly after WS therapy. Furthermore, the testis morphology of the WS-treated group indicated preserved germinal cells, active spermatogenesis, and absence of atrophy, vacuolation, and necrosis. **Conclusion:** WS treatment in ND-induced male rats alleviated the toxic effects of the ND on the gonads, probably due to its anti-inflammatory and antioxidant properties.

Keywords: Nandrolone decanoate, *Withania somnifera,* Gonadal toxicity, Testosterone, Sperm count, Sperm motility.

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INTRODUCTION

Anabolic Androgenic Steroids (AAS) are synthetic versions of testosterone. The usage of AAS among sports personnel and body muscle builders has significantly increased.[1] The prolonged usage of AAS Nandrolone Decanoate (ND) leads to gonadal toxicity among the male population. The therapeutic dose recommended for ND is 0.4 mg/kg/day. When the structure of ND has altered to produce the synthetic versions of AAS, and while exceeding the prescribed dose, it exhibits a strong anabolic effect but a weak androgenic effect.[2,3] The illicit use of AAS developed symptoms of altered cardiovascular, behavioral, and psychiatric characteristics. Androgens exhibit vital characteristics in the growth and functions of the male gonad system. The medical use of ND has resulted in the endocrine, cardiovascular, musculoskeletal, excretory, skin, and gastrointestinal disorders. The conventional therapy for gonadal toxicity may involve hormone replacement therapy, which may lead to other side effects affecting the vital organs of the body. The plant-based extracts may be helpful without any side effects on the biological system and cognitive activities. *Withania somnifera* (WS) belongs to the Solanaceae family, herbal drug with anti-oxidant, anti-inflammatory, anti-convulsant, cytoprotective, and adaptogenic characteristics. Earlier studies described the beneficial effects of *Withania somnifera* on Cardiovascular, Parkinson's disease, and Alzheimer's disease.[4]

Testosterone is a male reproductive hormone that aids in the growth of gonadal parts and their fertility. The synthetic derivative of male hormones has altered structural characteristics with enhanced anabolic nature and diminished androgenic properties. The sports personnel and adolescents depend upon AAS to boost their physical performance and tone their body physique, respectively.[5,6] The illicit users are not aware of the side effects that lead to damage of vital organs and tissues. The

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treatment for reversing the toxicity of the reproductive system caused by ND is tedious as the natural elimination of steroids from the body and testosterone replenishment should happen; otherwise, hormone replacement therapy needs to follow with caution of side effects. The treatment of gonadal toxicity was shown in earlier works using herbal drugs from various plant extracts. The Indian Ginseng or *Withania somnifera* (WS) was not tested to date for the treatment of male gonadal toxicity. The present study investigated the beneficial effects of water emulsion of WS extract (root powder) on ND-caused gonadal toxicity in male Wistar rats.

MATERIALS AND METHODS

Animals

The male rats were under incubation at the Biogen Laboratory Animal Facility, Bengaluru, India. It has facilitated to procure the male Wistar rats (*N*=24) that weighed (190-240 g), and all the rats were grown up at the Centre for Laboratory Animal Research (CLAR), Saveetha Institute of Medical and Technical Sciences, Chennai. The rats were sheltered in a temperature-controlled facility housing three per cage kept at a temperature of 25±2°C, humidity of 60-70%, and a natural light/dark cycle with free access to food and water ad libitum. All the animal procedures were approved by the Institutional Animal Ethics Committee (IAEC) of Saveetha Medical College (IAEC approval number: SU/CLAR/RD/004/2022). The animals were experimented according to the guidelines of the Committee for Control and Supervision of Experimentation on Animals (CCSEA, New Delhi, India).

Chemicals

The reagents and chemicals were procured from Sigma-Aldrich Inc., St Louis, Missouri, USA. ND (Deca-Durabolin, 4-oestren-17 β-ol-3-one-17-decanoate) (25 mg/mL) ampoules were from Cadila healthcare limited, Goa, India. Corn oil was from a local pharmacy; WS tablets (Ashwagandha) were purchased from the Himalaya Drug Company, Bengaluru, India. Other chemicals of analytical grade were purchased commercially. WS tablets impregnated with 250 mg of root extract were used to prepare the aqueous extract.

Experimental Design

The rats were separated into four groups (6 rats/groups) and studied as per the protocol discussed. The rats categorized as group I were injected with corn oil (1 mL per kg bw) intramuscularly in the thigh region twice weekly for four weeks and labeled vehicle control. The rats belonging to Group II were intramuscularly injected with ND (10 mg/kg bw) twice weekly in the thigh region for four weeks. Group III rats were injected with ND (10 mg/kg bw) twice weekly intramuscularly in the thigh region for four weeks and then administered with WS (200 mg/kg bw) once daily for four weeks. Group IV rats were intramuscularly injected with ND (10 mg/kg bw) twice weekly in the thigh region for four weeks and then administered with therapeutic drug WS (400 mg/kg bw) once daily for four weeks. The animals were given ND by intramuscular injections every Tuesday and Friday of the week.

The study rats were weighed before the start of the study and twice weekly during the rest of the experiment. After the termination of ND administration and therapeutic drug, the animals were under deep sedation using isoflurane, which was followed by blood sample collection at the retro-orbital plexus and then euthanized by cervical dislocation. After allowing the blood to clot for 20 min at room temperature, it was centrifuged (1000 g, 10 min), and the serum was extracted and preserved at -20°C for the study of biochemical parameters. The testes were excised, weighed, and fixed in 10% phosphate-buffered formalin (pH 7.4) for histopathological examination. The epididymis was excised and stored in neutral saline for semen analysis and cold storage (4°C) was maintained for sperm viability. Testosterone, malondialdehyde, catalase, and glutathione levels were experimented in adult male rats to study the therapeutic effects of WS root powder. The rats were administered with nandrolone decanoate for 30 days, followed by aqueous extract of WS root powder for 30 days as therapeutic intervention. The testes and seminiferous tubules were investigated for the analysis of various parameters.

Biochemical assays

Following the blood serum extraction, the Enzyme-Linked Immunosorbent Assay (ELISA) kit was used for testosterone measurements (Enzo Life Sciences, PA). We followed the protocols as described clearly by the manufacturer. An enzyme conjugate (200 µL) was mixed with 25 µL of serum per well and maintained at room temperature for 60 min. After incubation, the well-shaken mixture was rinsed with a wash solution (400 µL). The substrate solution (200 µL) was filled to each well and maintained at room temperature for 15min, and the reaction was stopped by adding a stop solution (100 µL). The absorbance level was experimented at 450 nm, and the results are indicated (ng/mL).

Assessment of oxidative stress and antioxidant levels

Lipid peroxidation assay was based on Yagi *et al.*, (1994) by measuring malondialdehyde levels. The thiobarbituric acid and serum sample was proportioned (2:1) by maintaining the temperature at 95°C for 15 min and allowed to cool. The placed test samples were centrifuged (1,000×g; 10 min) and observed at 532 nm.[7]

Glutathione (GSH) levels were experimented by following the method of Ellman *et al.*, (1964). The Ellman's reagent (3 mL) was added to a serum sample (20 µL) by incubating for 15 min; further, the absorbance level was experimented at 412 nm.[8] Catalase

(CAT) activity was determined according to Aebi *et al.* (1984). Furthermore, the serum test samples of (1.4 mL) were mixed with 0.1 mL PBS and 1.4 mL 20 mM H_2O_2 (1:4:5) and studied at 25°C within 2 min. The test mixture was then measured every 30 s on a tri-phase wavelength of 240 nm.[9]

$$E.U=(2.3/\Delta x)\,x\,(\log A1/\log A2)\,\text{activity} = U/L$$

Histological processing of the testes

The gonadal tissues were soaked in 4% buffered formalin (pH 7.4). After being dehydrated and fixed in paraffin, testicle sections were divided at a width of 5 µm and stained using Hematoxylin and Eosin (H&E). The deposition of excess stains over the slides were get rid of to prepare the permanent slides using a Dibutylphthalate Polystyrene Xylene (DPX) mount. The slides were studied using the microscope (Model: BX61 Olympus, Japan).

Actophotometer test

The spontaneous motor activity of the rat was experimented using the Actophotometer apparatus for 120 sec durations. The apparatus is a closed chamber with an optical transmitter and receiver. Whenever the receiver gets obstructed from the optical signals, the same accounts for the movement of rats.[10]

Statistical analysis

The data were studied using the Statistical Package for Social Sciences (SPSS) tool version 15.0, SPSS Inc, Chicago, Illinois, USA. Further, the graphical representation of data was done using Sigma plot tool version 14.5 (Systat Software Inc., San Jose, USA). One-way ANOVA test was performed to analyze the biochemical parameters, physical activity, whole body weight, and rat testes. The study data are represented as mean±SEM (n=6 each) and with the level of statistical significance set at $p \leq 0.05$. The data was analyzed by one-way ANOVA with Bonferroni 't' test for multiple comparison.

RESULTS

Measurement of body weight changes

The body weight of the male Wistar rats were assessed before delivering the ND dose, after ND dose induction, and after WS therapy among the control, ND, ND+WS 200, and ND+WS 400 groups, respectively (Table 1). The mean body weight of the ND group was 240.6±5.8g, 236.7±8.1 g, and 255.0±4.4 g, respectively whereas the body weight of the ND+WS 200 group was found to be 234.7±29.2 g, 226.8±15.7 g, and 291.0±9.8 g, respectively. The body weight of the ND+WS 400 group was 300.5±6.6 g, 263.7±7.5 g, and 296.2±9.0 g, respectively.

Biochemical assays

Table 2 represents the mean±SEM of the serum parameters like testosterone, malondialdehyde, glutathione, and catalase. After WS therapy, the testosterone level of control (5.58±0.07 ng/mL), ND (1.56±0.08 ng/mL), ND+WS 200 (4.09±0.11 ng/mL), and ND+WS 400 (4.47±0.24 ng/mL) group were experimented, respectively. The oxidative stress marker malondialdehyde indicated a significant increase among the ND group (8.22±0.17 nmol/mL) compared to the control group (5.04±0.02 nmol/mL). The values of MDA levels in WS treatment groups, ND+WS 200 (5.55±0.12 nmol/mL) and ND+WS 400 (5.20±0.04 nmol/mL) were significantly reduced ($p<0.000$). The activity of catalase enzyme in control, ND, ND+WS 200, and ND+WS 400 groups indicated values of 0.12±0.006, 0.07±0.004, 0.08±0.005, and 0.08±0.005 U/L, respectively. The mean glutathione levels of the control group showed increased levels (6.17±0.02 µmol/mL) when compared to the ND group (3.63±0.10 µmol/mL). In the ND+WS 200 group (5.37±0.12 µmol/mL) and ND+WS 400 group (5.47±0.11 µmol/mL) the levels were significantly higher compared to ND group.

Table 1: The effect of WS on nandrolone decanoate-induced body weight (g) changes in male Wistar rats.

Study Period	Group	Mean±SEM	Statistical Analysis
Initial (Before dose)	Control	312.5±3.5	
	ND	240.6±5.8	F=6.838
	ND+WS 200	234.7±29.2	p=0.002
	ND+WS 400	300.5±6.6	
After ND dose induction	Control	283.2±6.4	
	ND	236.7±8.1	F=6.437
	ND+WS 200	226.8±15.7	p=0.003
	ND+WS 400	263.7±7.5	
After WS therapy	Control	321.2±9.6	
	ND	255.0±4.4	F=10.346
	ND+WS 200	291.0±9.8	p=0.000
	ND+WS 400	296.2±9.0	

Gonad analysis

The sperm characteristics were assessed based on the sperm count and by estimating the sperm motility rate using a microscope at 20X magnification. The spermatozoa cell count was analyzed using a hemocytometer. The sperm cell count (9.02 X 10^6 cells/mL) and their motility rate (22.6%) decreased in the ND group than in the other study group; further, it was indicated by a decrease in the androgenic effect of the AAS as depicted in Table 2. The rate of motility of the spermatozoa cells was based on the equation

Rate of motility (%)=(The count of spermatozoa cells with advancing movements/200)x100

The weights of the left testis (2.19±0.23 g) and right testis (2.19±0.25 g) of the ND group were decreased significantly as compared to the control group; further, in the ND+WS200 group, both the left testis and right testis weights increased as compared to ND group. Furthermore, in the ND+WS400 group, the weights of left testis considerably decreased whereas right testis weights marginally increased as compared to ND group (Table 3).

Acto-photometer test

The physical movement of the rats was analyzed (Table 4) using the Acto-photometer instrument. The values of physical activity for the control, ND, ND+WS 200, and ND+WS 400 groups were 259±22, 258±37, 295±35, and 223±9 after ND administration. After WS therapy, the values were 174±13, 235±24, 193±27, and 143±10 for the respective groups.

Histopathological findings

The results of the control group as depicted in Figure 1 (a) demonstrate normal spermatozoa (green pointers), spermatocytes (red pointers), spermatids (red pointers), and mature spermatids (yellow pointers) in seminiferous tubules and Leydig cells in interstitial space (blue arrow), basement membrane (orange arrow). The ND group depicted in Figure 1 (b1, b2) showed increased interstitial spaces (blue arrow) and several spermatids with degenerative changes in seminiferous

Table 2: Evaluation of male fertility functions, oxidative stress, antioxidant, and hormone levels to test the efficacy of WS in ND induced.

Parameter	Group	Mean±SEM	Statistical Analysis
Testosterone (ng/mL)	Control	5.58±0.07	F=140.75
	ND	1.56±0.08	*p*=0.000
	ND+WS 200	4.09±0.11	
	ND+WS 400	4.47±0.24	
MDA (nmol/mL)	Control	5.04±0.02	
	ND	8.22±0.17	F=193.74
	ND+WS 200	5.55±0.12	*p*=0.000
	ND+WS 400	5.20±0.04	
Catalase (U/L)	Control	0.12±0.006	
	ND	0.07±0.004	F=24.362
	ND+WS 200	0.08±0.005	*p*=0.000
	ND+WS 400	0.08±0.005	
GSH (µmoL/mL)	Control	6.17±0.02	
	ND	3.63±0.10	F=126.850
	ND+WS 200	5.37±0.12	*p*=0.000
	ND+WS 400	5.47±0.11	
Spermatozoa Count (x10^6 cells/mL)	Control	25.05±0.09	
	ND	9.02±0.25	F=164.3
	ND+WS 200	14.4±0.69	*p*=0.000
	ND+WS 400	16.9±0.73	
Sperm Motility (%)	Control	71.8±0.6	
	ND	22.6±0.6	F=132.3
	ND+WS 200	45.9±1.7	*p*=0.000
	ND+WS 400	54.1±3	

Table 3: The protective effect of WS on Nandrolone decanoate-induced testes weights (g) changes in male Wistar rats.

Parameter	Group	Mean±SEM	Statistical Analysis
Left Testis	Control	2.93±0.20	
	ND	2.19±0.23	F=4.289
	ND+WS 200	2.36±0.11	*p*=0.017
	ND+WS 400	2.15±0.13	
Right Testis	Control	2.95±0.16	
	ND	2.19±0.25	F=4.300
	ND+WS 200	2.59±0.08	*p*=0.017
	ND+WS 400	2.22±0.15	

Table 4: The effect of WS on nandrolone decanoate-induced locomotor activity in male Wistar rats.

Parameter	Group	Mean±SEM	Statistical Analysis
Actophotometer (After ND Induction period)	Control	259±22	
	ND	258±37	F=1.089
	ND+WS 200	295±35	*p*=0.377
	ND+WS 400	223±9	
Actophotometer (After WS therapy period)	Control	174±13	
	ND	235±24	F=3.646
	ND+WS 200	193±27	*p*=0.003
	ND+WS 400	143±10	

tubules (green arrow) as indicated by atrophy (yellow arrow), necrosis (black arrow), vacuolation, and decreased number of spermatogenic cells. The ND+WS 200 group showed preserved germinal cells which is depicted in Figure 1 (c); furthermore, it demonstrated smaller seminiferous tubules with larger interstitial space (yellow arrow mark), which indicated partial improvement of the active spermatogenesis (green arrow mark). The ND+WS 400 group revealed mild degenerative changes in seminiferous tubules with significant improvement of active spermatogenesis (orange arrow), absence of atrophy, vacuolation, and necrosis (Figure 1 (d)).

DISCUSSION

The present study demonstrated the dose-dependent therapeutic efficacy of aqueous WS root powder on ND-induced adverse effects on male reproductive organs, which was consistent with the study reported by Hallak *et al.*, (2020).[11] The safety and toxicity profile is the prime concern before employing any plant extract for treatment. Hence, previous studies reported no adverse toxicity of WS root powder extract at all tested doses (up to 2000 mg/kg of body weight).[12] The present study used one-tenth and one-fifth of the reported maximum non-toxic dose (WS 200 mg/kg and WS 400 mg/kg of 2000 mg/kg) to study the potential effects of WS against ND toxicity on male gonads of Wistar rats.

The study results revealed a significant decrease in the whole-body weight of the ND group against the control group following the ND injections. The decreased body weight of the rats could be due to the increase in body metabolism facilitated by burning stored fat. The WS-treated rats were under sedentary activity to cause body weight gain at the end of the WS therapy period. The present result was similar to Giri (2016) in that the body weight gain was significant after the WS therapy to rats compared to the non-drug induced rats. The study reported that an increase in body weight could be due to the restoration of the absorption capacity of the intestine.[13]

The ND group showed a significant decrease in the testosterone levels compared to control group. Further, WS therapeutic groups (ND+WS200 and ND+WS400) showed a significant improvement in testosterone levels compared to the ND group as depicted in Figure 2 (a). This trend indicated that ND has spurious effects on the male reproductive system, similar to Shahraki *et al.*, (2015) study.[14] Mohammed *et al.*, studied the protective effect of *Cynara scolymus* Leaf Extract (CLE) to alleviate the side effects of ND; there was a decrease in the testis weight in the ND+CLE group compared to the control group. Furthermore, the testosterone levels did not increase significantly after CLE therapy.[15] The present work indicated significant increase in testes weight of the rats after WS treatment among the ND+WS 200 group and ND+WS 400 group. The oxidative stress marker malondialdehyde

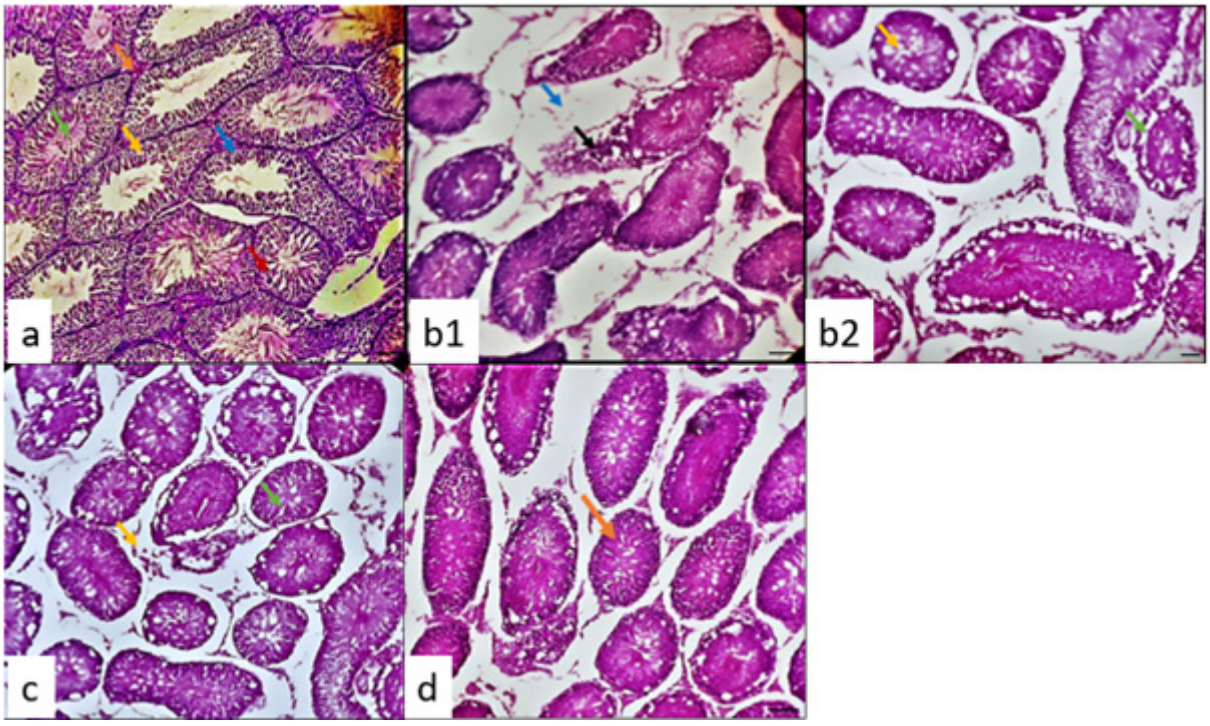

Figure 1: (a) The histological study of the testicular region of the control group depicting the spermatozoa (green arrow), spermatocytes (red arrow), spermatids (red arrow), and mature spermatids (yellow arrow) in seminiferous tubules and Leydig cells in interstitial space (blue arrow), basement membrane (orange arrow). (b1 & b2) The histology of the testes of the ND group depicting large interstitial spaces (blue arrow) and several spermatids with degenerative changes in seminiferous tubules (green arrow) as indicated by atrophy (yellow arrow), necrosis (black arrow), vacuolation and decreased number of spermatogenic cells. (c) The histology of the tested ND+WS 200 treated group depicts preserved germinal cells and small seminiferous tubules; large interstitial spaces (yellow arrow mark) were identified along with partial improvement of active spermatogenesis (green arrow mark) (d) The histology of the testes of the ND+WS 400 treated group depicts mild degenerative changes in seminiferous tubules with better improvement of active spermatogenesis (orange arrow), absence of atrophy, vacuolation, and necrosis. Scale bar: 100µm. Magnification: 20X.

(MDA) showed a significant increase among the ND-induced rats compared to healthy control rats. This high level indicates the severe oxidative damage caused by ND to the gonadal organs. The level of MDA in WS treated rats were significantly lesser compared to ND group as shown in Figure 2(b). The ill effect of MDA was reduced following WS treatment to ND-induced rats with 200 mg/kg and 400 mg/kg doses, respectively. Taken altogether, the water emulsion of WS root powder has indicated a significant decrease in MDA levels. Saddick *et al.*, (2021) studied the effect of ND-induced oxidative stress on rats, and the results of their study regarding testes weight and MDA were similar to the present study with decreased testes weights and increased MDA concentration among the ND-induced group.[16] The antioxidant Glutathione (GSH) showed a significant decrease among the ND-induced rats compared with the healthy group. Further, there was an increase in the GSH level in the treatment group as compared to the ND group as depicted in Figure 2(c). The serum catalase levels did not change significantly among the ND and WS-treated groups. The serum catalase did not show a significant statistical difference among the study group as depicted in Figure 2(d). Catalase and glutathione are antioxidant enzymes that aid in the reduction of reactive oxygen species. The ND group showed

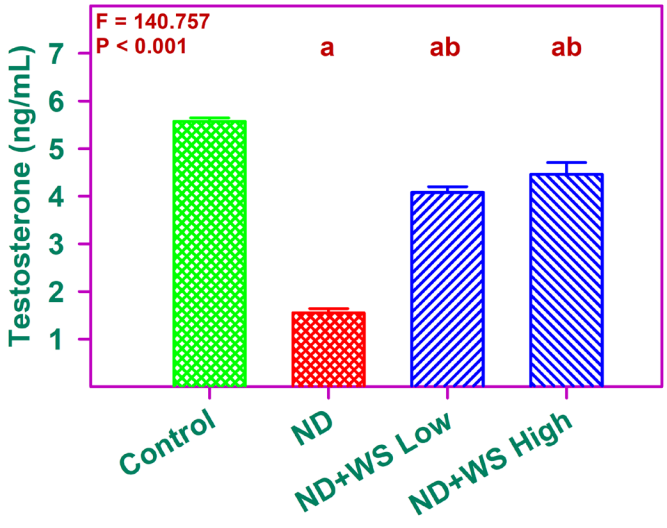

Figure 2 (a): The bar graph shows the effect of (WS 200 mg/kg) and (WS 400 mg/kg) doses on Nandrolone (ND) induced changes of testosterone level in rats.

decreased catalase whereas the WS treatment groups showed a marginal increase in catalase levels, however GSH levels were significantly improved.

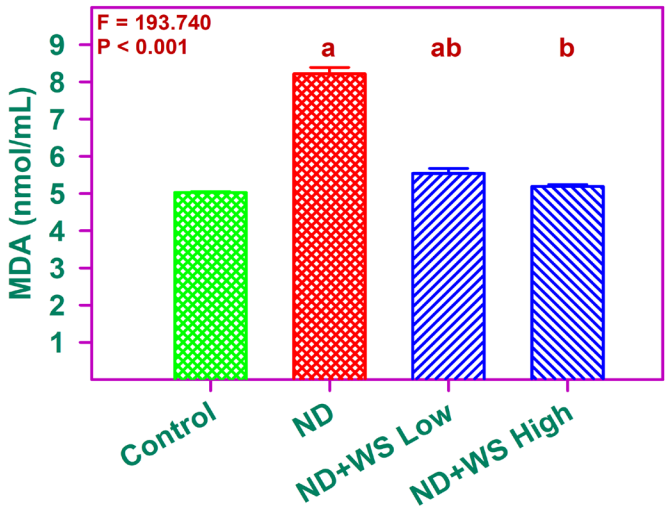

Figure 2 (b): The bar graph shows the serum malondialdehyde levels among the study groups.

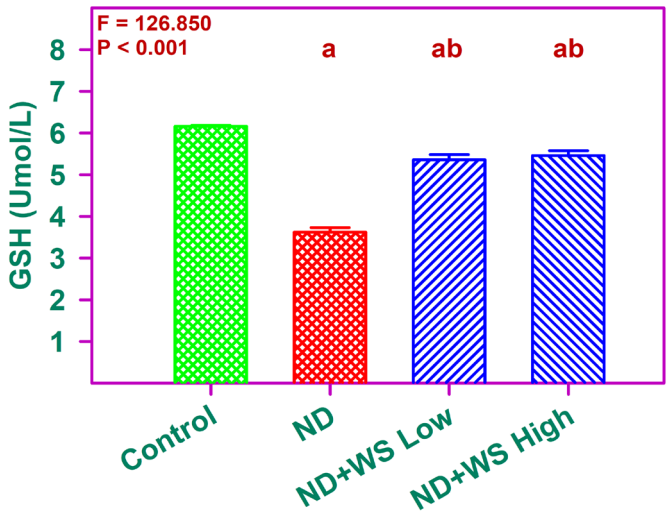

Figure 2 (c): Quantitative analysis of testicular glutathione among the study groups.

The present study has an interesting observation, described in Table 3. The testes of the ND+WS 200 group showed a significant increase in their weights compared to the ND+WS 400 group; the increase in testes weights in the ND+WS 200 group could be due to increased locomotor activity observed against the ND+WS 400 group. Moreover, the whole-body weight of ND+WS 400 group was comparatively higher than the ND and ND+WS 200 group which have resulted in decreased testes weights. The results were similar to Stretonovic *et al.*, (2021) study in which ND induced rats were subjected to swimming activity. Thereby, the testicle weight was increased by 36%.[17]

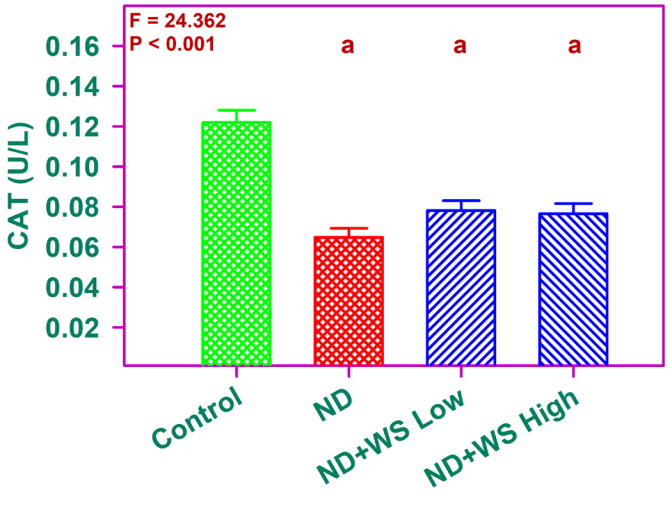

Figure 2 (d): The bar graph depicts values of testicular catalase among the study groups.

The number of spermatozoa depicted in Figure 3 (a) and their rate of motility shown in Figure 3 (b) was decreased significantly in the ND group; the WS-treated group showed significant recovery from the damage caused by ND in both the sperm count as well as their motility rate. Based on the photoactometer study observed before treatment, the physical activity was higher in the ND and the drug intervention group. After thirty days of treatment, the locomotor activity of the control group was decreased significantly due to increased body weight at the end of the study.

Sherif *et al.* studied the testicular damage caused by methotrexate. After treatment with *Ginko biloba* (GIN) leaf extract, MDA was reduced by 39% compared with the methotrexate (MTX) group. The oxidative damage caused by methotrexate has reduced with less improvement in the testosterone levels compared with the control group. The treatment efficacy of this plant in GIN+MTX group was 3 ng/mL which is lesser than ND+WS200 (4.09 ng/mL) and ND+WS400 group (4.47 ng/mL) in the present study.[18] Razak *et al.*, have studied the anti-oxidant properties of *Aquilaria malaccensis* (AM) against Cyclophosphamide (CP) toxicity in which the absolute testicular weight did not show any significant difference in the CP treated group as well the therapy group compared to the control group. Furthermore, the number of spermatozoa in the CP+AM-500 group has resulted in $3.39X10^{7}$/mL which is comparative less than the present study group ND+WS400 group ($16.9X10^{9}$/mL), and their motility rate (50.8%) was near similar compared to the present study (51%).[19] The results are similar to Ismail *et al.*, (2018), where the rats did not undergo any regular strenuous exercise like swimming or roto rod activity.[20,21] In the present study, the measured testes weight of the ND group decreased when compared to the control group; this could be due to the influence of the hypothalamic-pituitary-gonadal axis that affects testicular apoptosis.[22]

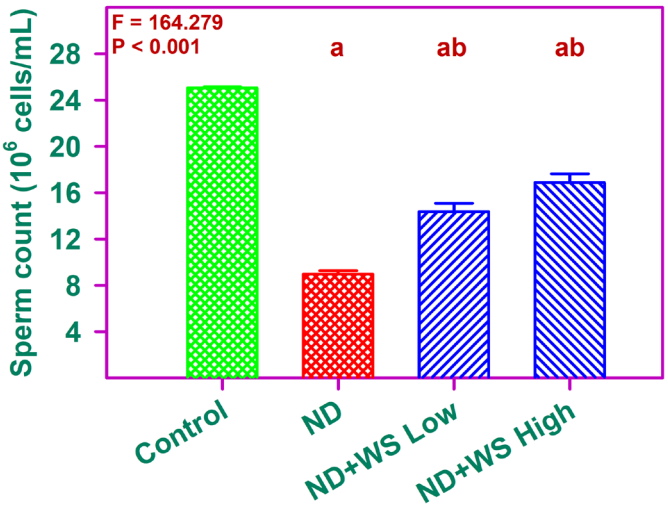

Figure 3 (a): Efficacy of different doses of *Withania somnifera* on sperm count analysis in ND induced gonadal toxicity.

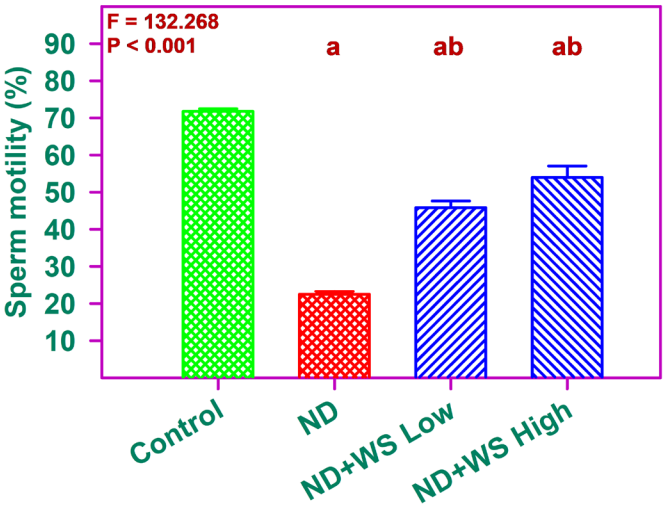

Figure 3 (b): Dose dependant effect of *Withania somnifera* on sperm motility rate in ND induced gonadal toxicity.

The histopathological study of the control group testes indicated the presence of spermatozoa, spermatocytes, spermatids, and Leydig cells. The source of testosterone production and the development of sperm into mature ones are healthy in the control group. The ND-induced group has indicated large interstitial spaces with decreased spermatogenic cells. Furthermore, the degenerative changes in the seminiferous tubules, atrophy, and necrotic state were similar to earlier studies.[23-25] This could be due to the decreased production of testosterone and local ischemia of the testicles. The effect of AAS may reduce blood perfusion to the reproductive organs.

Adjei *et al.*, (2023) studied the effect of *Mucuna pruriens* (Mp) seed powder at different doses (500 mg/kg, 1000 mg/kg, and 2000 mg/kg). The non-healthy body weight of the rats was increased significantly in all the groups after 90 days of dosage. The testosterone increase was not significant after 90 days of dosage. Further, the sperm motility was comparatively less (low dose: 50%, medium dose: 57%, and high dose: 56%) than the WS study. The present study using 200 mg/kg and 400 mg/kg of WS extract indicated a significant increase in testosterone.[26] Jannatifar *et al.*, (2015) studied the supraphysiological dose of Nandrolone decanoate among mature and immature rats. The severe depletion of sperm cells significantly decreased Sertoli cells, testis size, and the diameter of seminiferous tubules.[27] Langade *et al.*, (2023) studied the effect of *Withanone* (win) on the sub-acute liver toxicity. Win is a characteristic of withanolide with toxcophores that could cause antagonistic drug reactions when taken in excess of the prescribed dose limit.[28]

Baghel *et al.*, (2023) observed the reduced activity of Superoxide Dismutase (SOD) in the pancreas, brain, and testes of diabetic mice (Streptozotocin STZ). The catalase activity was increased following administration of *Withaferin-A* (WA) to rats and the values of WA+STZ group was higher than the STZ group.[29] Bentaiba *et al.*, (2023) studied lead-induced damage on reproductive organs and reported decreased testosterone levels and sperm count. On administration of *W. frutescens*, the rats exhibited no toxic effects; furthermore, the testosterone level and sperm count also significantly increased.[30] The treatment groups showed mild deteriorations in seminiferous tubules and significant improvement in active spermatogenesis. Furthermore, the therapeutic effect of WS has resulted in the absence of atrophy, vacuolation, and necrosis of the testicular cells. The mean weights of the testes were decreased in the ND group, showing ND's adverse effects on the reproductive system. Altogether, it is well evidenced that WS root extract has potential therapeutic efficacy on reproductive disorders.

CONCLUSION

ND acts as a temporary energy booster for sustained physical activity. The significant damage of ND on the male gonads is obvious; further, the usage of the same is continued either by athletes or body builders for a long duration, and it may cause irreversible damage to the reproductive organs. To summarize, the WS regimen has significantly reversed the ill effects of ND as evidenced from our study investigations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AAS: Anabolic Androgenic Steroids; **AM:** *Aquilaria malaccensis*; **ANOVA:** Analysis of Variance; **CAT:** Catalase; **CLAR:** Centre for Laboratory Animal Research; **CLE:** *Cynara scolymus* leaf extract; **CP:** Cyclophosphamide; **ELISA:** Enzyme-Linked Immunosorbent Assay; **GSH:** Glutathione; **H&E:** Hematoxylin and eosin; **IAEC:** Institutional Animal Ethics Committee; **MDA:** Malondialdehyde; **ND:** Nandrolone decanoate; **PBS:** Phosphate-buffered saline; **SEM:** Standard Error Mean; **SPSS:** Statistical Package for Social Sciences; **WS:** *Withania somnifera*.

SUMMARY

The present-age adolescents have started using Anabolic Androgenic Steroids (AAS) to develop an attractive physique and to perform extended sports activity with long-lasting energy duration through known or unknown side effects. The present study has revealed the impact of Nandrolone Decanoate (ND) on the male reproductive system of rats; ND has caused a decrease in the Testes weight, sperm count, and motility. In the present scenario, there is no remedial treatment to reverse the damage caused to the testes. Hence, the investigated root extracts of *Withania somnifera* (WS) demonstrated significant improvement in spermatogenesis and amelioration of gonadal toxicity. Therefore, individuals suffering from sexual dysfunction and other reproductive disorders can be intervened with WS for no side effects.

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