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Withania somnifera ameliorates nandrolone-decanoate-induced brain damage in rats by inhibiting cell death, prodynorphin mRNA expression and acetylcholinesterase activity

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The misuse of anabolic-androgenic steroids by athletes and non-athletes causes harmful effects on the central nervous system. In Ayurvedic medicine, *Withania somnifera* (WS) as an herbal drug has been reported for several functions including adaptogenic, anticonvulsant, cytoprotective and antioxidant. The present study investigated the neuroprotective functions of WS (100, 200 and 400 mg/kg body weight) in nandrolone decanoate (ND)-induced (16 mg/kg body weight) brain injury in male Wistar rats. ND was injected intramuscularly twice weekly for 4 weeks. The water emulsion of WS root powder was administered orally once daily for 30 days to ND-treated rats. At the end of the experiment, anxiety-like behaviour was assessed in rats using the elevated plus maze. Haematoxylin-and-eosin-stained coronal sections of the parietal cortex and hippocampus of ND rats showed severe alterations in brain histology compared with control rats. Acetylcholinesterase (AChE) activity in the striatum and prodynorphin gene expression in the hippocampus was significantly elevated in the ND group compared with the control group. Treating ND induced rats with various doses of WS significantly reversed the brain damage, anxiety behaviour, increased striatal AChE activity and reduced prodynorphin gene expression in the hippocampus. In conclusion, WS extract can be used as a neuroprotective agent to reduce the effects of anabolic steroids.

Keywords: Acetylcholinesterase, Anabolic-androgenic steroids, Nandrolone decanoate, Necrosis, Striatum, *Withania somnifera*

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Anabolic androgenic steroids (AASs) are synthetic derivatives of the male gonadal hormone testosterone and are used clinically and illegally[1]. They are employed for the treatment of male hypogonadism, burns, surgery, trauma, anaemia, radiation therapy, HIV and metastatic breast tumors[2-4]. However, due to their beneficial role in tissue building, maintenance of muscle mass and strength, they have been widely used by adults, adolescents and athletes in their attempt to strengthen their muscle development and to promote recovery[5]. The therapeutic dose of ND recommended varies from 50 to 100 mg per week for women and 100 to 200 mg per week for men[6]. An individual who takes a dose that is 10-100 fold higher than the standard dose can be subjected to many adverse effects[7]. AAS abuse is implicated with psychiatric symptoms such as mania, depression, aggression and the development of dependence; behavioural changes like increased irritation; acute psychosis and anxiety[8].

The striatum is a part of the brain that contains myriad cholinergic pathways and clinical evidence suggests that excessive AAS concentrations may damage the cholinergic system, which executes several vital functions like learning, memory and the organization of movements[9]. The Acetylcholinesterase (AChE) hydrolyses the neurotransmitter acetylcholine (ACh) in cholinergic synapses as well as in neuromuscular junctions; this action rapidly terminates the transmission of nerve impulses[10]. The hippocampus area of brain which is implicated mainly in learning and memory functions of rat has a relatively high density of androgen receptors, implicating a possible link between cognitive function and androgen receptors[11]. AASs induce hippocampal apoptosis and inhibit hippocampal neurogenesis, suggesting that AASs could reduce hippocampal

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volume, which could be a basis for the AAS-associated spatial memory impairments that have been observed in human and animal studies[12]. Previous studies have shown that administration of the AAS nandrolone decanoate (ND) elevated the prodynorphin messenger RNA (mRNA) level in male rat hippocampus and also affected the dynorphinergic system by altering the level of dynorphin B[13]. Other works support plausible correlations between impaired memory and dynorphin levels, and researchers suggest that the dynorphinergic system modulates learning and memory[14]. Gonadal hormones exhibit intricate role in cognitive processes, and thus it is conceivable that cognitive functions are influenced by AASs[15]. AASs appear to upregulate androgen receptors in the hippocampus, cerebral cortex and hypothalamus[16]. Long-term AAS abusers are characterized by a high level of anxiety and extreme mood swings[17]. Chronic infusion of high doses of ND induces anxiolytic behaviour and impairs social memory, spatial learning and recall performance via., activation of central androgen receptors[18]. There is only limited data on the impact of ND in the CNS.

Withania somnifera Linnaeus Dunal (Family Solanaceae), hereafter WS, is a notable herb in Ayurveda, Indian traditional medicine[19]. WS has adaptogenic, antistress, anticonvulsant, cytoprotective, neuroprotective and antioxidant properties[20]. Earlier works have demonstrated the protective effect of WS on age-related neurodegenerative diseases like Parkinson's and Alzheimer's diseases[21]. WS reduces the levels of AChE activity in the brain of scopolamine induced amnesic mice[22]. The cognitive and psychomotor performance was improved following WS extract intake in healthy human participants implicating increased cortical muscarinic ACh receptor capacity. This ability might partly explicate the cognition-enhancing and memory-improving functions of WS extracts in animals and in humans[23]. In addition, WS treatment can reduce anxiolytic activity[24]. Our recent work revealed the therapeutic benefits of a water emulsion of WS root powder on ND-induced biochemical alterations and hepatorenal toxicity[25]. However, the protective and beneficial function of WS in ND-induced brain injury is not known. The present study investigated the neuroprotective potentials of WS on ND-induced injury to the parietal cortex, striatum and hippocampus of brain and assessed anxiety-like behaviour in mice.

Materials and Methods

Animals

Thirty-six male Wistar rats (180-250 g) were acquired from Biogen Laboratory Animal Facility (Registered Lab Animal Breeders, Bangalore, India) and maintained at the Centre for Laboratory Animal Research (CLAR) facility of the institution. The animals were housed three per cage in a controlled environment with free access to food and water *ad libitum* at a temperature of 25±2°C, humidity 40%-60% and a natural light/dark cycle. All animal procedures were approved by institutional animal ethics committee (IAEC) of Saveetha Medical College (IAEC number: SU/CLAR/RD/004/2018). Animal experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA, New Delhi, India).

Materials

The reagents and chemicals used in this study were obtained from Sigma Aldrich Chemical Company, St. Louis, MO, U.S.A Primer Premier software, Premier Biosoft, USA was used for the design of primers and also confirmed with the Primer-BLAST tool from the National Center for Biotechnology Information (NCBI) website. All the primers were synthesized by Sigma Genosys (Bangalore, India). M-MuLV Reverse Transcriptase and RNase Inhibitor were purchased from Fermentas (Thermo Scientific), USA The list of primers used is shown in Table 1.

Drug treatment

WS tablets (Himalaya Drug Company, Bangalore, India) were used in this study; each tablet contained 250 mg of root extract and was prepared in distilled water. ND (decadurabolin or 4-oestren-17β-01-3-one-17-decanoate) was purchased from Cadila Health Care Ltd. (Goa, India; 100 mg/mL ampules). ND was diluted in corn oil.

The randomly segregated rats were divided into five groups: (n = 6 each for the ND+WS100 and ND+WS400 groups; n= 8 each for the other groups)

Table 1 — Primers used for reverse transcription-polymerase chain reaction amplification

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product size (bp)
Prodynorphin	ATGGCGTGGTCCAGGCTGATGC	AGTTTGTAGATTTAGAAGCCTTATCC	400
β-actin	CATCTCTTGCTCGAAGTCCA	ATCATGTTTGAGACCTTCAACA	162

Group 1: Vehicle control (C; n = 8) – Rats injected with 1 mL corn oil/kg body weight intramuscularly (i.m.) in the lateral aspect of thigh twice weekly for 4 weeks.

Group 2: ND (n = 8) – Rats received 16 mg/kg ND (i.m. in 1 mL corn oil/kg body weight) in the lateral aspect of thigh twice weekly for 4 weeks.

Group 3: ND+WS 100 (n = 6) – Rats received 16 mg/kg ND (i.m. in 1 mL corn oil/kg body weight) twice weekly for 4 weeks and oral WS extract (100 mg/kg body weight) once daily for 30 days.

Group 4: ND+WS 200 (n = 8) – Rats received 16 mg/kg ND (i.m. in 1 mL corn oil/kg body weight) twice weekly for 4 weeks and oral WS tablets (200 mg/kg body weight) once daily for 30 days.

Group 5: ND+WS400 (n = 6) – Rats received 16 mg/kg ND (i.m. in 1 mL corn oil/kg body weight) twice weekly for 4 weeks and oral WS tablets (400 mg/kg) once daily for 30 days.

The animals were weighed before the beginning of the experiment and on days 11, 21 and 31 of the experiment. A week after the last ND injection, the animals were anaesthetised with isoflurane and sacrificed by cervical dislocation. The brain was carefully dissected, washed in ice-cold saline and weighed. The parietal cortex, hippocampus and striatum were excised. The parietal cortex and hippocampus were used for haematoxylin and eosin (H&E) staining, the striatum was used to evaluate AChE activity and the hippocampus was used to examine gene expression.

Histopathological evaluation

The parietal cortex and hippocampus were fixed in 10% phosphate-buffered formalin (pH 7.5), processed by routine histological methods and embedded in paraffin blocks. Five micrometer thick coronal sections were cut with a microtome. All sections were stained with H&E and viewed under a light microscope (Olympus iNEA, CH20iBIMF, Olympus Opto Systems India Pvt. Ltd, Noida, India).

AChE activity

AChE activity was assessed in homogenates of striatum following a modification of the method reported by Srikumar *et al*[26]. The mixture was set by mixing 0.4 mL of the homogenate with 2.6 mL phosphate buffer (0.1 M, pH 8.0) and 100 µL of Elman's reagent (270 µM). This mixture was then pre-incubated for 2 min at 30ºC and the reaction was triggered with the addition of 20 µL Acetylthiocholine (30 mM). The product of thiocholine reaction with Elman's reagent was measured every 2 min for 10 min at 412 nm. The absorbance per minute was measured. The specific activity is expressed as µmol ACTC/min/mg protein.

Reverse transcription polymerase chain reaction (RT-PCR)

Bilateral hippocampi were collected from each rat and placed on an ice plate. Fifty to hundred milligrams of tissue were treated with 1 mL pre-cold TRIzol (Thermo Fischer Scientific, USA) and then sufficiently ground with a tissue grinding appliance. The homogenates were then incubated on ice for 5 min. Next, added 0.2 mL chloroform to the homogenates and were drastically agitated for 15 sec and then incubated on ice for 2-3 min. After completing the centrifugation step at 12,000 *g* for 15 min, the supernatant was removed and mixed with 0.5 mL isopropanol and incubated at room temperature for 5-10 min. This solution was subsequently centrifuged at 12,000 *g* for 10 min at 4°C and the supernatant carefully removed. The pellet was washed with 0.5 mL of freshly made 75% ethanol and centrifuged at 6,300 *g* for 5 min at 4°C. The supernatant was then discarded and the pellet was dried at room temperature and then dissolved in water with 1 g/L diethyl pyrocarbonate (DEPC) for a final concentration of 0.1 µg/µL RNA.

An ultraviolet spectrophotometer was used to measure the RNA concentration and quality (using a 10-20-fold dilution). The absorbance at 260 nm was used to determine the concentration (µg/µL) and the 260/280 ratio was used to determine the purity (required to be 1.8-2.0). The RNA integrity was confirmed by agarose gel electrophoresis, observing the 28S, 18S and 5S bands on a gel stained with ethidium bromide using a gel imaging system (ProGen Gel Doc system, Progen Scientific store, London, UK). Five micrograms of total RNA were uncoiled by heating at 70°C for 10 min and then reverse transcribed into complementary DNA (cDNA) in a 20 µL reaction in a sterile nuclease-free tube. Each reaction contained 1 µL Oligo (dT) primer, 7.5 µL DEPC-treated water, 4 µL 5X reaction buffer, 0.5 µL (20 U) RNase Inhibitor, 2 µL of a 10 mM dNTP mix and 2 µL (40 U) M-MuLV Reverse Transcriptase. The mixture was mixed gently and centrifuged briefly. It was incubated at 37°C for 1 h, followed by 70°C for 10 min to stop the reaction. A portion of the cDNA (2 µL) was amplified in a 50 µL reaction volume containing 4.0 µL of the prodynorphin forward primer (2 µM), 4.0 µL of the

prodynorphin reverse primer (2 μM) (Sino biological, China), 3.0 μL of the β-actin forward primer (2 μM), 3.0 μL of the β-actin reverse primer, 5.0 μL 10X buffer, 5.0 μL of a 2 mM dNTP mix, 0.5 μL *Taq* DNA polymerase (5 U/μL), 2.0 μL template and 23.5 μL sterile distilled water. PCR amplification was carried out in an Eppendorf Mastercycler® ep (Eppendorf AG, Hamburg, Germany). The thermal cycling conditions were: initial denaturation at 94°C for 5 min; 34 cycles of 94°C for 40 s, 65°C for 60 s and 72°C for 60 s and a final extension at 72°C for 10 min. The amplified products were separated on a 1.0% agarose gel in 1X Tris–Borate–ethylene-diaminetetraacetic acid (TBE) at 75 V for 3 h. The gel was stained with ethidium bromide and the amplified product was visualized and photographed on a ProGen Gel Doc system.

Elevated plus maze (EPM)

The day after final ND injection, the rats were subjected to EPM test for 3 min to evaluate the level of anxiety. The maze consists of two perpendicular open arms (50 × 10 cm) without side walls and two closed arms (50 × 10 × 50 cm) extending horizontally at right angles from a central area (10 × 10 cm). The maze was elevated to a height of 50 cm above the ground. At the beginning of the experiment, the rat was placed in the centre of the EPM facing the open arm and allowed to freely explore the maze for 3 min. The parameters analyzed were as follows: open and closed arms entries in numbers, the time spent exploring the closed and open arms, the percentage of time spent in the open arm and the number of entries into the open arms. An arm entry is counted when the animal enters the arm with all four paws.

Statistical analysis

We performed one-way analysis of variance (ANOVA) for the data analysis. The values are presented as mean ± standard error of the mean (SEM). Differences between the means of groups were analysed by the Student–Newman–Keuls method. $p < 0.05$ was considered statistically significant.

Results

Brain weight

Brain weight results showed no difference between the control group and the treatment groups and the values were not statistically significant (Table 2, $p= 0.906$).

Table 2 — The effect of WS and ND on the brain weight of male Wistar rats

Group	%
Control	0.881 ± 0.0924
ND	0.768 ± 0.0810
ND+WS 100	0.762 ± 0.0256
ND+WS 200	0.767 ± 0.0690
ND+WS 400	0.836 ± 0.199

The values are presented as the per cent of body weight (mean ± standard error of the mean; n = 6 rats per group). There was no difference between the groups (one-way analysis of variance, p=0.906). Abbreviations: ND, nandrolone decanoate; WS, *Withania somnifera*.

Histopathological evaluation

Histopathological evaluation of the parietal cortex of the control group revealed normal pattern of morphology of neurons with all neuronal cell types such as the pyramidal cells, stellate cells and granule cells with distinct nuclei (Fig. 1a). In the ND group, there were immature cells and hyperchromatic dead neurons (seen at lower magnification; data not shown), apoptotic neurons with pyknotic nuclei (Fig. 1b). In the ND+WS100 group (Fig. 1c), there were perineural cells (seen at lower magnification; data not shown), neuroglial cells, pyramidal cells and fewer pyknotic nuclei compared with the ND group. In the ND+WS200 group (Fig. 1d), there was normal architecture with compactly arranged granule cells with rounded pale vesicular nuclei; the hilum comprised large pyramidal cells with long processes, astrocytes and microglial cells. In the ND+WS400 group (Fig. 1e), there was almost normal neuronal architecture with less neurodegeneration, and prominent nuclei compared with the ND group.

The histopathological changes noticed in the hippocampus are shown in Figure 2. In the control group, there were normal neurons in the CA1, CA2 and CA3 regions with three distinct layers, namely polymorphic, pyramidal and ganglion (Fig. 2a). By contrast, in the ND group (Fig. 2b), there was neuronal loss, a thin dentate gyrus with sparse and scattered hilar cells in the granular layer with numerous immature and apoptotic neuronal cells, cell shrinkage and nuclear condensation. In addition, the thickness of the CA1, CA2 and CA3 neuronal cell layers was remarkably decreased due to severe neurodegeneration. In the ND+WS100 group (Fig. 2c), nuclear condensation was less severe compared with the ND group. In the ND+WS200 group (Fig. 2d), nuclei were less condensed and

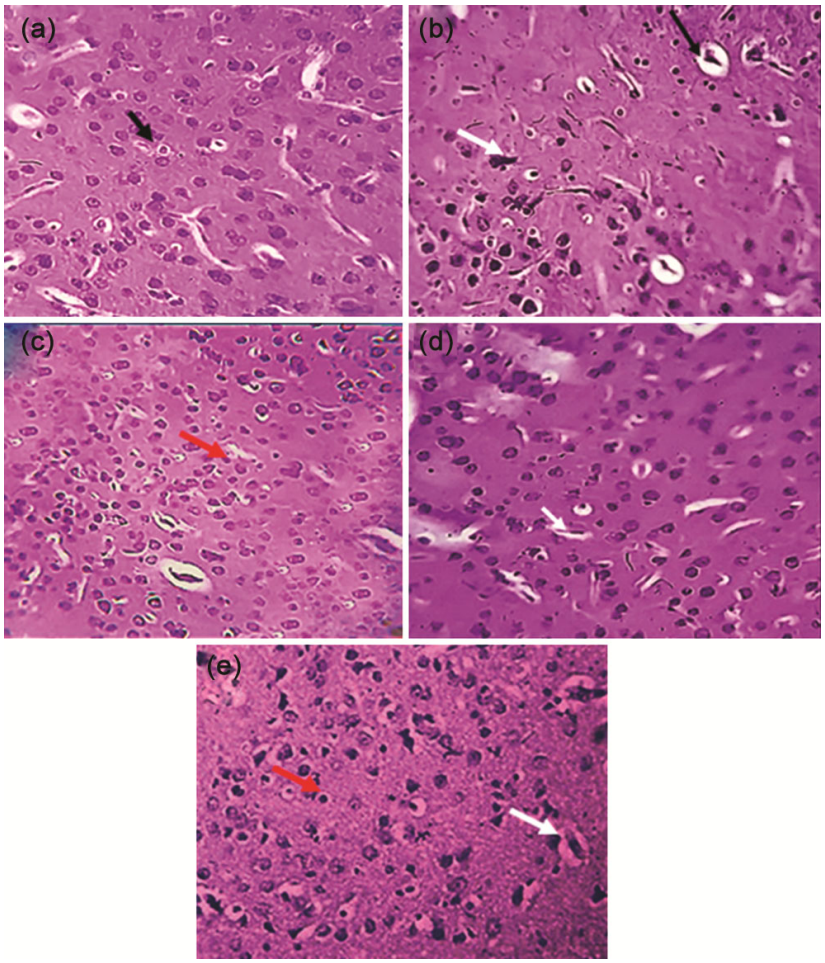

Fig. 1 — Photomicrographs of parietal cortex region of brain stained with H&E. The pictures of the Control (a), ND (b), ND + WS100 (c), ND + WS 200 (d) and ND + WS 400 (e) groups are shown respectively. Red arrow – neuroglial cells; long black arrow- apoptotic neurons; white arrow- pyramidal cells; small black arrow- normal neuronal cells. Magnification 100x.

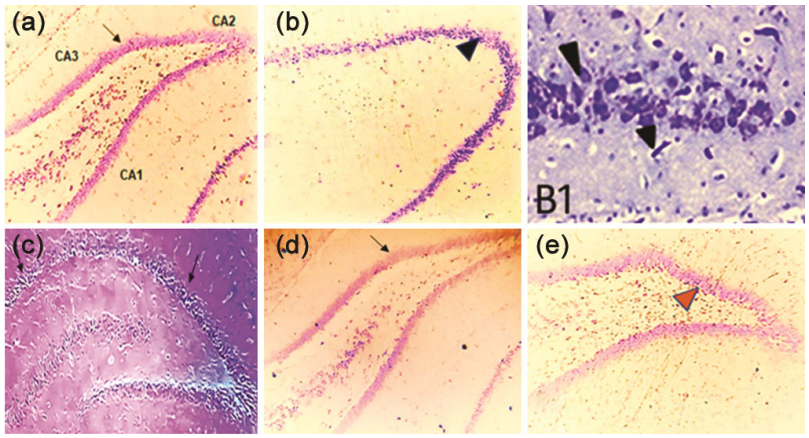

Fig. 2 — Photomicrographs of brain hippocampal region stained with H&E. (a) Control rats showed normal neurons with CA1, CA2 and CA3 regions (arrow mark) in hippocampus. (b) ND group showed neuronal loss, cell shrinkage and nuclear condensation (black arrow head) with numerous immature and apoptotic neuronal cells. Panel B1 shows higher magnification (100x) of ND group with arrow head depicting apoptotic neurons with pyknotic nuclei. (c) ND+WS100 group showed thin Dentate gyrus, granule cell layer in Dentate gyrus containing sparse and scattered few hilar cells (arrow marks).(d) In ND+WS200 group, nucleus was less condensed with minimal damage (black arrow mark) (e) In ND+WS400 group, nuclear condensation was more minimized, cell shrinkage was dramatically reduced (orange arrow head). All images captured at 20x magnification except panel B1.

tissue damage was reduced compared with the ND group. In the ND+WS400 group (Fig. 2e), there was markedly less nuclear condensation and cell shrinkage compared with the ND group.

AChE activity in the striatum

Figure 3 shows the AChE activity measurements in the striatum. The values (mean ± SEM, in µmol ACTC/mg protein) were: control = 0.333 ± 0.009; ND = 2.49 ± 0.117; ND+WS100 = 2.55 ± 0.142; ND+WS200 = 0.924 ± 0.117; and ND+WS400 = 1.39 ± 0.113. The AChE activity of the ND group increased by 86.6% compared with the control; the average value is beyond the reference range for rats. ND+WS200 and ND+WS400 groups showed significantly reduced AChE activity compared with the ND group (62% and 44%, respectively, $p<0.001$). No significant difference was noticed between the ND and ND+WS100 AChE activity ($p=0.704$). Considering the WS treatment groups, the ND+WS200 group showed more protection than the ND+WS100 and ND+WS400 groups ($p<0.05$).

Prodynorphin mRNA expression in the hippocampus

The prodynorphin mRNA level in the hippocampus was significantly higher in the ND compared with the control group (Fig. 4, $p<0.05$). Compared with the ND group, the prodynorphin mRNA level in the ND+WS200 and ND+WS400 was significantly reduced ($p<0.05$). There was no difference between the ND and ND+WS100 groups.

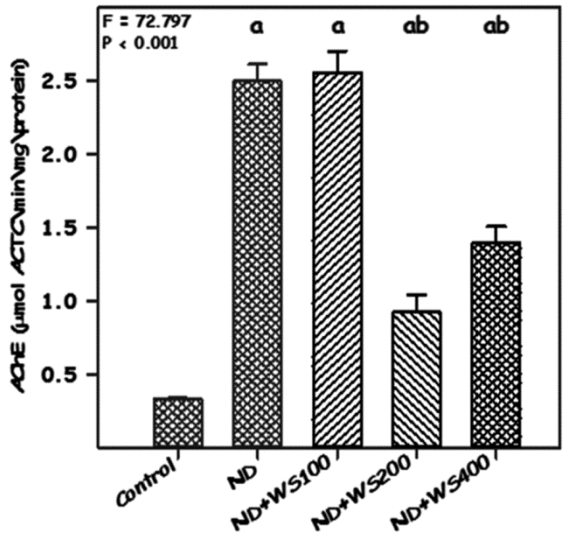

Fig. 3 — AchE activity measurements in brain striatum. The values present the mean ± standard error (n = 6 each group). $p< 0.001$ [a]Significantly different from the control group; [b]Significantly different from the ND group

EPM analysis

As shown in the Table 3, during the 3 min exploration of maze, the comparison between rats of the ND group and control group didn't reveal any significant difference in the total time spent in the centre of EPM despite a marginal increase seen in ND group and it was not statistically significant (C=7.125±2.799, ND=31.000±15.450; $p=0.112$). The comparison between ND and WS treatment groups did not reveal any significant difference in the total time spent in the centre of EPM (ND= 31.000±15.450, ND+WS100= 35.000±6.78, ND+ WS200=33.86 ± 13.04, ND+WS400=5.000±1.7; $p=0.112$). The rats of ND group showed significant decrease in the total time spent in the closed arms compared with control and WS treatment groups (C=172.62±2.8, ND=123.50±14.8ND+WS100= 135.2±10.58, ND+WS200=143.8 ± 17.8, ND+WS400= 173.00 ± 2.16; $p=0.015$). This was found to be statistically significant ($p=0.015$). The rats of the ND group significantly increased the total time and the frequency in the open-arm entries compared to the control group of rats (C= 0.000±0.000, ND= 43.250±17.866; n=8, $p=0.019$), while the

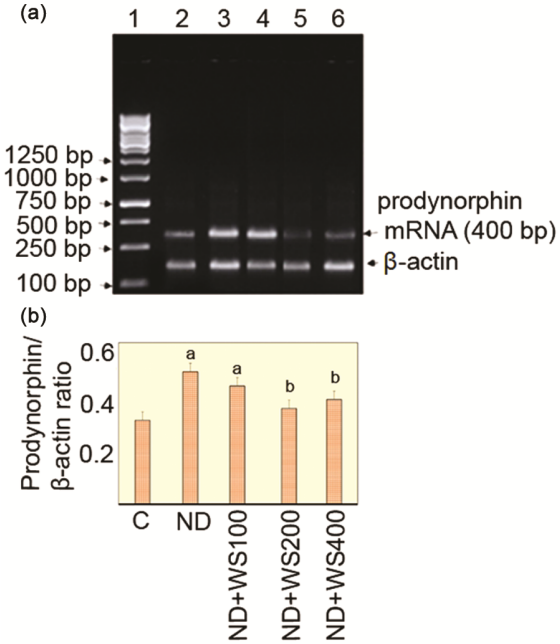

Fig. 4 — Gel picture showing the Prodynorphin gene expression analysis in hippocampal region of brain (A). Lane 1 - marker, lane 2 - Control, lane 3 - ND, lane 4 - ND + WS100, lane 5 - ND + WS200, lane 6 - ND + WS400. The relative gene expression (prodynorphin/β-actin ratio) in various groups is shown as bar graph (B). The values present the mean ± standard error (n = 4). $p< 0.05$ [a]Significantly different from the control group; [b]Significantly different from the ND group

Table 3 — Elevated plus maze results to assess the effect of WS on ND toxicity

Parameter	Group	Time (s)	p
	Control	7.125 ± 2.799	
	ND	31.000 ± 15.450	
Centre	ND+WS100	35.000 ± 6.78	0.112
	ND+WS200	33.86 ± 13.04	
	ND+WS400	5.000 ± 1.7	
	Control	172.62 ± 2.8	
	ND	123.50 ± 14.8	
Closed	ND+WS100	135.2 ± 10.58	0.015
	ND+WS200	143.8 ± 17.8	
	ND+WS400	173.00 ± 2.160	
	Control	0.000 ± 0.000	
	ND	43.25 ± 17.87	
Open	ND+WS100	10.17 ± 7.44	0.019
	ND+WS200	10.00 ± 4.614	
	ND+WS400	2.000 ± 2.000	

Each rat spent 180 s in the elevated plus maze. The values indicate the time spent in the maze centre and closed and open arms; they are presented as the mean ± standard error of the mean (n = 6 rats for the ND+WS100 and ND+WS400 groups; n = 8 for all other groups). The p values are based on one-way analysis of variance. Abbreviations: ND, nandrolone decanoate; WS, *Withania somnifera*.

ND+WS groups of rats showed similar open-arm entries as such of the control group (ND+WS100 = 10.17±7.441, ND+WS200 = 10.000±4.614, ND+ WS400= 02±02, p> 0.05).Thus more time in the open arms was spent by ND group compared to the control group, while the time spent by ND+WS groups in the open arms was similar to the control group. The parameters such as time spent and frequency on the open arms of the EPM test were increased in ND-treated rats imparting an anxiolytic-like effect of ND.

Discussion

Several seminal studies have shown that ND cause brain damage and associated complications due to steroid-induced effects[27,28]. The neurological complications associated with ND include abnormal and aggressive behaviour, apart from defective neurotransmission due to changes in brain chemicals such as dopamine, serotonin and noradrenaline[29,30]. While several therapeutic interventions have been attempted in the form of synthetic drugs and exercise to treat these drug-induced adverse effects[31-33], there is a lack of studies that have used herbal medicine to overcome the side effects of ND. Therefore, there is an urgent need to manage ND complications through identification of safe drugs to ameliorate such adverse effects of ND.

In previous work, we have shown the possible beneficial and protective effects of WS from ND toxicity through the evaluation of various biochemical and histopathological (liver and kidney) parameters[25,34]. In the present work, we have generated concrete evidence to support the brain protective functions of WS through behaviour (EPM), brain histopathology, striatal AChE activity and prodynorphin gene expression in the hippocampus. Indeed, this is the first report with mechanistic evidence to substantiate the potential of WS to counteract ND-mediated effects through the assessment of brain prodynorphin mRNA expression. The present findings also provide new insights into the neuromodulatory functions of WS through assessing AChE activity in the striatum. Earlier works demonstrated that chronic nandrolone toxicity induced behavioural impairments in rats that involved anxiolytic-like-behaviour[35,36], memory dysfunction, and aggressive behaviour with altered levels of brain monoamines, ACh, lipid peroxidation, tumour necrosis factor and elevated AChE activity[32].

The present EPM results clearly showed that a high dose of ND induced aggressive behaviour. The mechanism involved in ND-mediated aggressive behaviour is glutamate-induced *N*-methyl-D-aspartate receptor (NMDAR) hyperexcitability and decreased clearance of extracellular glutamate content[29]. The fact that more time spent by ND group rats in the open arm during the EPM test implicates an anxiolytic effect due to aggressive and hyperactive behaviour. However, treating rats with various doses of WS root extract countered the ND-induced anxiolytic and hyperactive behaviours. Activation of central androgenic receptors (ARs) has been implicated to mediate anxiolytic behaviour, impaired social memory, spatial learning and recall performance following chronic high doses of ND[37]. Earlier studies have proved the beneficial role of WS in cognitive functions using various models of memory dysfunctions that involve AChE inhibition, neurodegeneration, inflammation and oxidative stress[38,39]. Therefore, the neuroprotective potentials of WS could be well substantiated based on other research works as well as the key findings of the present investigation. ND is believed to provoke a cascade of inflammatory and apoptotic events that involve oxidative injury – including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), B-cell lymphoma 2 (Bcl-2), second mitochondria-derived activator of caspases/direct

IAP-binding protein with low PI (SMAC/DIABLO) and vesicular monoamine transporter 2 (VMAT2) – to induce brain cell degeneration[40]. The present histopathological results provide plausible evidence to support the ability of WS extract to reverse those degenerative changes in the parietal cortex and hippocampus, which could be through inhibition of inflammation, apoptosis and oxidative stress[41,42]. The ability of WS root extract to reverse aggressive and hyperactive behavioural abnormalities could be attributed to its adaptogenic functions and pharmacological efficacy in stress management[43-44]. The phytochemical profile of WS has been intensively explored[45] which implicates therapeutic value of this neuroprotective drug.

Conclusions

The present work has demonstrated the neuroprotective functions of WS root powder against ND-induced toxicity. Specifically, WS extract ameliorated histopathological abnormalities in several brain regions, reduced AChE activity in the striatum, increased prodynorphin mRNA expression in the hippocampus and counteracted anxiolytic and hyperactive behaviour. These findings emphasize that WS extract could be a viable and promising protective agent to ameliorate the anabolic steroid induced brain damage.

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Conflict of interest

The authors have no conflicts of interest to declare.

Authors' contributions

SS and VJ conceived the ideas of research, SS designed the experiments, SSV and SS performed experiments, generated data and analyzed results.

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