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ORIGINAL ARTICLE

Protective Effect of *Withania somnifera* on Nandrolone Decanoate-Induced Biochemical Alterations and Hepatorenal Toxicity in Wistar Rats

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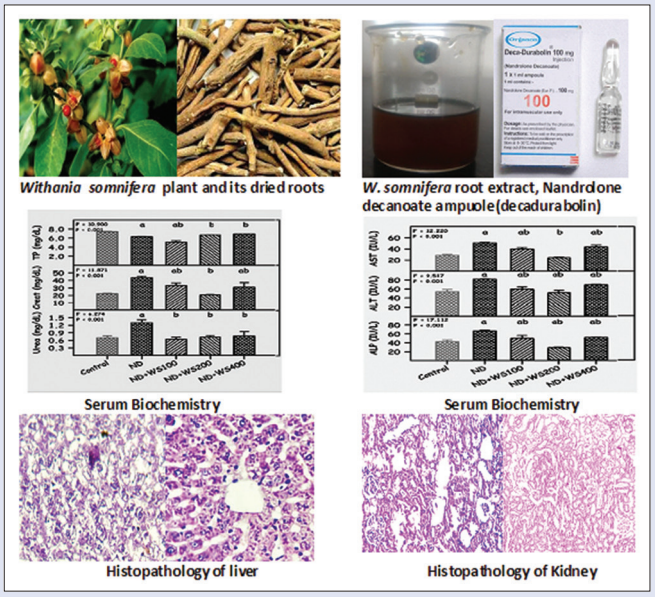
ABSTRACT

Background: Despite the beneficial effects of anabolic-androgenic steroids in the treatment of osteoporosis, hypogonadism, bone mineralization, and some muscle-wasting disorders, its misuse by athletes and non-athletes causes hepatorenal toxicity. *Withania somnifera* (WS) is a very important herb in Ayurveda and has adaptogenic, anticonvulsant, cytoprotective, and antioxidant properties. **Objectives:** The main objective of the study is to investigate the protective effect of WS also known as Ashwagandha on nandrolone decanoate (ND)-induced liver and kidney injury in Wistar rats using biochemical and histopathological assessment. **Materials and Methods:** Group 1 – control rats received corn oil intramuscularly, Group 2 – ND group received 16 mg/kg of ND intramuscularly, Group 3 (ND+WS100), Group 4 (ND+WS200), and Group 5 (ND+WS400) were treated with water emulsion of WS root powder (100 mg/kg, 200 mg/kg, and 400 mg/kg) along with ND (16 mg/kg). All ND treatments were given twice weekly for 4 weeks. WS was diluted in distilled water and administered orally once daily for 30 days. Liver marker enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, total protein (TP), creatinine, and urea levels were evaluated followed by histopathological assessment of liver and kidney. **Results:** ND group showed elevated levels of liver enzymes, TP, creatinine, urea, and severe alterations in the hepatic and renal histology as compared with control. Drug treatments with WS to ND group considerably reversed liver marker enzymes, TP, creatinine, urea levels, and pathological damage caused to liver and renal tissue. **Conclusion:** The study findings reveal the protective role of WS on ND-induced hepatorenal toxicity implicating its antioxidant and therapeutic functions.

Key words: Hepatotoxicity, nandrolone decanoate, necrosis, nephrotoxicity, rats, *Withania somnifera,* therapeutic agent

SUMMARY

- Hepatorenal toxicity was induced in rats by intramuscular injection of anabolic steroids such as nandrolone decanoate at a dose level of 16 mg/kg twice weekly for 4 weeks. The serum levels of liver marker enzymes (alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase), creatinine, and urea levels were significantly increased in nandrolone decanoate-administered rats apart from significant histopathological changes of liver and kidney tissues which are an indication of hepatorenal toxicity. The nandrolone decanoate-induced rats when subjected to drug treatment with *Withania somnifera* (100, 200, and 400 mg/kg) it strikingly reverted the serum levels of liver marker enzymes, creatinine, and urea and also protected the liver and kidney tissues from ND-induced damage. Thus, it can be concluded that *Withania somnifera* root holds potential to ameliorate anabolic steroid-induced hepatorenal toxicity.



Abbreviations used: WS: *Withania somnifera*; ND: Nandrolone decanoate; AAS: Anabolic-androgenic steroid; ANOVA: Analysis of variance; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TP: Total protein; Creat: Creatinine; SEM: Standard Error of Mean.

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INTRODUCTION

Anabolic-androgenic steroids (AASs) are synthetic derivatives of male hormone testosterone which are used clinically and illegally.[1] They are used in the treatment of hypogonadism, burns, surgery, trauma, osteoporosis, anemia, HIV, and metastatic breast tumors.[2] However, due to its beneficial properties such as tissue building booster, maintenance of muscle mass, strength and bone health, it has been widely used by

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the adults, adolescents, and athletes in their attempt to increase their strength for athletic performance and bodybuilding, to accelerate muscle development, and to promote recovery.[3] The recommended therapeutic dose of AAS, nandrolone decanoate (ND) in humans varies from a dose of 50–100 mg/week for women and 100–200 mg/week for men.[4] When an individual intends to take 10–100 folds higher than the therapeutic dose which is a misuse, it can cause many adverse effects such as myocardial infarction, cholestasis, adenoma, hepatocellular carcinoma, and nephrotoxicity.[5,6] ND, also called as Deca-durabolin, is considered Worldwide as one of the most widely abused AAS.[7] In an animal study, it was clearly depicted that administration of anabolic steroid, ND in rats affected the cellular redox homeostasis in tissues such as liver, kidney, and heart leading to oxidative stress.[8]

Withania somnifera (WS), commonly known as "Ashwagandha" or Indian ginseng, is a well-known medicinal plant from Solanaceae family and has a major role in the treatment of liver diseases.[9] WS is one of the important medicinal plants in the Ayurvedic system of Medicine.[10] WS is a small erect evergreen shrub of four to five feet in height and is cultivated in different parts of India, Nepal, and Africa. WS has anti-inflammatory, immunomodulatory, antiarthritic, and antiapoptotic functions.[11] It has proven its antioxidant and free-radical scavenging activity in various disease models.[12] Its hepatoprotective effect in paracetamol-induced liver injury was also reported.[13] Evidences support hepatoprotective and nephroprotective functions of WS root extract against various chemical agents by inhibition of oxidative stress and cytoprotective mechanisms.[14,15] Withanolide-rich fraction has proven hepatoprotective effect in acetaminophen toxicity in rats via inhibition of tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1-β), cyclooxygenase-II, and inducible nitric oxide synthase (iNOS), thereby implicating its anti-inflammatory and antioxidant potentials.[16] Several studies have shown that WS root extract reversed heavy metal-induced oxidative stress complications.[17,18] Neuroprotective and immunomodulatory role of WS roots could also be witnessed through many published works.[19-23] However, there are no studies to report the effect of WS in ND-induced biochemical and histopathological alterations. Hence, in the present study using Wistar rats, the beneficial effect of WS was explored in ND-induced biochemical alterations, as well as liver and kidney injury.

MATERIALS AND METHODS

Drugs and reagents

ND (Deca-Durabolin, 4-oestren-17 β-ol-3-one-17-decanoate) was purchased from Cadila Healthcare Ltd., India (100 mg/mL ampuoles), corn oil was purchased from local pharmacy, Chennai, WS tablet (Ashwagandha) was purchased from Himalaya Drug Company, India. Other chemicals of analytical grade were purchased commercially.

Animals

Thirty-six male Wistar rats weighing 180-250g, procured from Biogen Laboratory Animal Facility (Registered Lab Animal Breeders), Bangalore, and maintained in Centre for Laboratory Animal Research, Saveetha Institute of Medical and Technical Sciences, Chennai, were used. The animals were housed three per cage in a controlled environment with free access to food and water *ad libitum*, room temperature of 25°C ± 2°C, humidity 40%–60%, and natural light/dark cycles. All animal procedures were approved by the Institutional Animal Ethics Committee of Saveetha Medical College, Chennai (IAEC-SU/CLAR/RD/004/2018), and animal experiments were performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA, New Delhi, India).

The animals were randomly divided into five groups (n = 6 each for ND+WS100 and ND+WS400 groups; others 8 each per group).

- Group 1: Control[C] – Rats received 1 mL/kg body weight of corn oil intramuscularly (i. m) in the thigh region twice weekly for 4 weeks as vehicle
- Group 2: ND group – Rats received intramuscular (i. m) injection of ND (16 mg/kg) in the thigh region in a volume of 1 mL/kg body weight twice weekly for 4 weeks
- Group 3: ND+WS100 mg/kg treated group (ND+WS100) – Rats received i. m injection of ND (16 mg/kg) in a volume of 1 mL/kg body weight twice weekly for 4 weeks and WS drug-treated orally (100 mg/kg body weight) once daily for 30 days
- Group 4: ND+WS200 mg/kg treated group (ND+WS200) – Rats received i. m injection of ND (16 mg/kg) in a volume of 1 mL/kg body weight twice weekly for 4 weeks and WS drug-treated orally (200 mg/kg body weight) once daily for 30 days
- Group 5: ND+WS400 mg/kg treated group (ND+WS400) – Rats received i. m injection of ND (16 mg/kg) in a volume of 1 mL/kg body weight twice weekly for 4 weeks and WS drug-treated orally (400 mg/kg) once daily for 30 days. ND was diluted in corn oil. WS tablets were used in this study, and each tablet contained 250 mg extract of WS root dissolved in distilled water.

The animals were weighed before the start of the experiment and on days 11, 21, and 31 during the experimental period. The animals were given ND by intramuscular (i.m) injections every Monday and Wednesday and four days after the last doses of injection, all groups of animals were anesthetized by isofluorane, and blood was collected by retroorbital plexus and then sacrificed by cervical dislocation. After allowing the blood to clot for 30 min at room temperature, it was centrifuged (1000 g, 10 min), and serum was collected and stored at −20°C for biochemical analysis. Liver and kidney were excised and washed in ice-cold saline, weighed, and fixed in 10% phosphate-buffered formalin (pH 7.4) for histopathological examination.

Biochemical analysis

The levels of serum enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), total protein (TP), creatinine, and urea levels were estimated. All the above estimations were carried out using semi-autoanalyzer (Robonik Prietest) as per the standard protocols.

Histopathological assessment

Liver and kidney tissues were fixed in 10% phosphate-buffered formalin (pH 7.4) and processed by routine histological methods and embedded in paraffin blocks. Five micrometer thick sections were cut coronally. All sections were stained with hematoxylin and eosin and examined under light microscope (Olympus iNEA).

Statistical analysis

All data were analyzed by one-way ANOVA. The values are presented as mean ± Standard error of mean. The mean value differences between the groups were analyzed by Student–Newman–Keuls method. P <0.05 was considered as statistically significant. Sigma plot 13 (Systat software) was used for the statistical analysis.

RESULTS

The ND+WS100 group rats showed significant increase in the body weight at the end of the experiment [Table 1]. This indicates that there was a significant increase of body weight (146.63 ± 7.00 g; P = 0.001) in the ND+WS100 treatment group in comparison with control, ND, ND+WS200, and ND+WS400 groups which is quite interesting. The comparison between ND and control group did not reveal any significant difference with the body weight despite a marginal decrease of body

weight seen in ND (108.00 ± 4.27; P = 0.084). The ND+WS200 and ND+WS400 treatment groups did not reveal any significant difference in the body weight [122.68 ± 8.10 g, 109.43 ± 7.03 g; P = 0.531; Table 1] when compared with control and ND groups [Table 1].

There were no significant changes noticed in the liver and kidney weight of rats between the control and treated groups (ND, ND+WS100, ND+WS200, ND+WS400) in this study [Table 2, P = 0.862, P = 0.077].

Biochemical studies showed significant increase in liver enzymes, e.g., AST, ALT, ALP as well as creatinine, urea, and significant decrease in TP levels in ND group when compared to the control group. However in WS-treated groups, ND induced changes of all liver marker enzyme levels as well as TP, creatinine, and urea were significantly reversed [Figures 1 and 2, P < 0.001].

Table 1: The effect of nandrolone decanoate on body weight (%) in male Wistar rats

Group	Days	Mean±SEM	Statistical analysis
Control	1	100±0.00	F=3.131
	11	103.78±2.15	P=0.041
	21	108.98±3.35	
	31	119.76±8.85	
ND	1	100±0.00	F=2.453
	11	102.76±1.87	P=0.084
	21	107.57±2.69	
	31	108.00±4.27	
ND+WS100	1	100±0.000	F=17.194
	11	129.36±4.33*	P=0.001
	21	141.18±5.75*	
	31	146.63±7.00*	
ND+WS200	1	100±0.00	F=3.421
	11	108.62±3.59	P=0.031
	21	117.79±6.28	
	31	122.68±8.10	
ND+WS400	1	100±0.00	F=0.757
	11	106.51±3.91	P=0.531
	21	109.69±6.53	
	31	109.43±7.03	

Values are expressed as mean±SE. Based on the one-way ANOVA, there is a *Statistically significant difference from the respective control group (P<0.05). ND: Nandrolone decanoate; WS: *Withania somnifera*; SE: Standard error; SEM: Standard error of mean

Table 2: The effect of nandrolone decanoate on liver and kidney weight in male Wistar rats

Parameter	Group	Mean±SEM	Statistical analysis
Liver weight	Control	3.80±0.49	F=0.321
	ND	3.68±0.35	
	ND+WS100	3.87±0.16	P=0.862
	ND+WS200	3.77±0.23	
	ND+WS400	4.402±0.92	
Kidney weight	Control	1.45±0.13	F=2.349
	ND	1.94±0.15	
	ND+WS100	1.78±0.13	P=0.077
	ND+WS200	1.98±0.15	
	ND+WS400	1.84±0.16	

Values are expressed as mean±SE. Based on one-way ANOVA with Student-Newman-Keul's multiple comparison test, there is not a statistically significant difference (P=0.862, P=0.077) for liver and kidney weight between the control and all treatment groups. ND: Nandrolone decanoate; WS: *Withania somnifera*; SE: Standard error; SEM: Standard error of mean

The AST levels of control, ND, ND+WS100, ND+WS200, and ND+WS400 are 28.4 ± 1.8, 50.9 ± 0.98, 39.6 ± 2.8, 24.06 ± 1.2, and 43.6 ± 03.14 IU/L, respectively. The AST levels of ND group increased by 78.8% as compared with control, and these changes are beyond the reference range of rat species. However, in ND+WS-treated groups, the increase in the AST level was lesser than the ND group. In comparison with ND group, there was 22.13%, 52.7% and 14.26% decrease of AST levels in ND+WS100, ND+WS200, and ND+WS400 group, respectively. This shows there was significant decrease of AST levels in the drug-treated groups (ND+WS100, ND+WS200, and ND+WS400) in comparison with ND (P < 0.001). For the comparisons between various WS-treated groups, ND+ WS200 group showed more protection than ND+WS100 and ND+WS400 group (P < 0.001).

The ALT levels of control, ND, ND+WS100, ND+WS200, and ND+WS400 are 53.6 ± 5.1, 81.9 ± 0.58, 59.2 ± 5.3, 51.3 ± 5.7, and 69.1 ± 1.7 IU/L, respectively. The ALT levels of ND group increased by 52.6% as compared with control, and these changes are beyond the reference range of rat species. However, in ND+WS-treated groups, the increase in the ALT level was lesser than the ND group. In comparison with ND group, there was 42.3%, 56.9%, and 23.8% decrease of ALT levels in ND+WS100, ND+WS200, and ND+WS400 groups, respectively. This shows that there was significant decrease of ALT levels in the drug-treated groups (ND+WS100, ND+WS200, and ND+WS400) in comparison with ND (P < 0.001). For the comparisons between various WS-treated groups, ND+ WS200 group showed more protection than ND+WS100 and ND+WS400 groups (P < 0.001).

The ALP levels of control, ND, ND+WS100, ND+WS200, and ND+WS400 are 42.4 ± 3.8, 66.9 ± 1.6, 49.5 ± 6.9, 29.1 ± 1.3, and

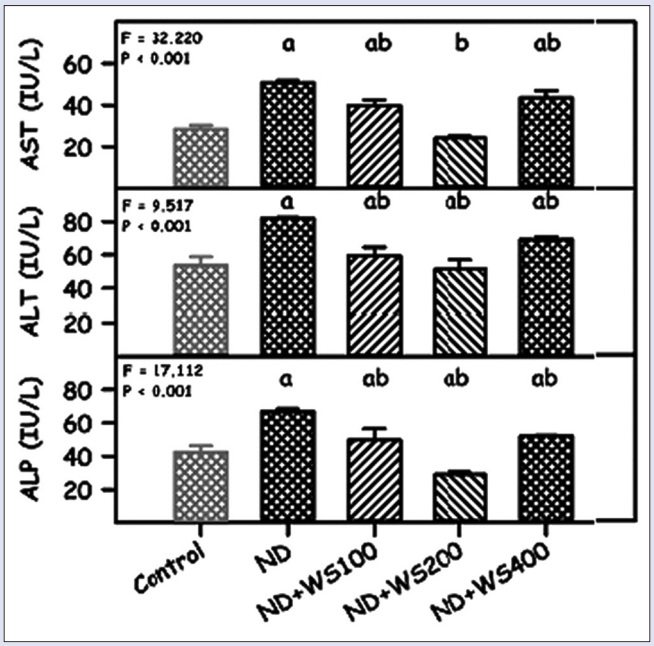

Figure 1: The protective effect of *Withania somnifera*, 100 mg/kg (*Withania somnifera* 100), 200 mg/kg (*Withania somnifera* 200), and 400 mg/kg (*Withania somnifera* 400) against nandrolone decanoate-induced changes in serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Values are mean ± Standard error (n = 6 each for *Withania somnifera* 100 and *Withania somnifera* 400; others 8 each). The "F" and "P" values are by one-way ANOVA with Student–Newman–Keul's multiple comparison test, [a]Significantly different from control group, [b]Significantly different from nandrolone decanoate group

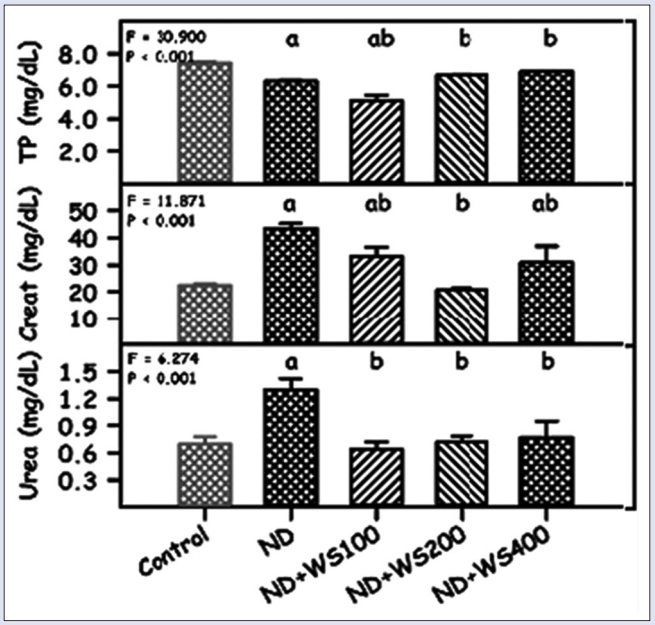

Figure 2: The protective effect of *Withania somnifera*, 100 mg/kg (*Withania somnifera* 100), 200 mg/kg (*Withania somnifera* 200), and 400 mg/kg (*Withania somnifera* 400) against nandrolone decanoate-induced changes in serum total protein, creatinine (creat), and urea. Values are mean ± Standard error (n = 6 each for *Withania somnifera* 100 and *Withania somnifera* 400; others 8 each). The "*F*" and "*P*" values are by one-way ANOVA with Student–Newman–Keul's multiple comparison test, [a]Significantly different from control group, [b]Significantly different from nandrolone decanoate group

51.9 ± 0.8 IU/L, respectively. The ALP levels of ND group increased by 57.8% as compared with control, and these changes are beyond the reference range of rat species. However, in ND+WS-treated groups, the increase in the ALP level was lesser than the ND group. In comparison with ND group, there was 40.9%, 89.3%, and 35.5% decrease of ALP levels in ND+WS100, ND+WS200, and ND+WS400 groups, respectively. This shows there was significant decrease of ALP levels in the drug-treated groups (ND+WS100, ND+WS200, and ND+WS400) in comparison with ND ($P < 0.05$). For the comparisons between the WS-treated groups, the ND+WS200 group showed more protection than ND+WS100 and ND+WS400 groups ($P < 0.001$).

The TP levels of control, ND, ND+WS100, ND+WS200, and ND+WS400 are 7.4 ± 0.06, 6.3 ± 0.09, 5.06 ± 0.037, 6.6 ± 0.04, and 6.9 ± 0.04 g/dL, respectively. The TP levels of ND group decreased by 15.1% as compared with control, but it was within the normal reference range of rat species. However, in ND+WS200 and ND+WS400-treated groups, the decrease in the TP level was lesser than the ND group. In comparison with ND group, there was 5.19%, 8.08% increase of TP levels in ND+WS200 group and ND+WS400 groups, respectively. This shows that there was significant increase of TP levels in the drug-treated groups (ND+WS200 and ND+WS400) in comparison with ND ($P < 0.001$). The TP level of ND+WS100 group decreased by 16.6% when compared to control, but it was within the normal reference range of rat species.

The creatinine levels of control, ND, ND+WS100, ND+WS200, and ND+WS400 are 22.4 ± 0.65, 43.5 ± 1.7, 33.2 ± 3.39, 20.6 ± 0.61, and 30.9 ± 6.03 mg/dL, respectively. The creatinine levels of ND group increased by 93.9% as compared with control, and these changes are beyond the reference range of rat species. However, in ND+WS treated groups, the increase in the creatinine content level was lesser than the ND group. In comparison with ND group, there was 46.2%, 100%, and 56.29% decrease of creatinine levels in ND+WS100, ND+WS200 group, and ND+WS400 groups, respectively ($P < 0.001$). For the comparisons between various WS-treated groups, ND+ WS200 group showed more protection than ND+WS100 and ND+WS400 groups ($P < 0.001$).

The urea levels of control, ND, ND+WS100, ND+WS200, and ND+WS400 are 0.69±0.08, 1.3 ± 0.13, 0.63 ± 0.08, 0.72 ± 0.07, and 0.77 ± 0.18 mg/dL, respectively. The urea levels of ND group increased by 86.2% as compared with control and such changes are beyond the reference range of rat species. However, in ND+WS treated groups, the increase in the urea levels was lesser than the ND group. In comparison with ND group, there was 95%, 82.7%, and 75.8% decrease of urea among ND+WS100, ND+WS200, and ND+WS400 groups, respectively ($P < 0.001$). This shows that there was significant decrease in the levels of urea in the drug-treated groups (ND+WS100, ND+WS200, and ND+WS400) in comparison with ND ($P < 0.001$). For the comparisons between various WS treatment groups, it did not reveal any significant difference with the urea levels ($P = 0.860$, $P = 0.77$).

Histopathological evaluation of liver of control group rats showed normal hepatic cellular architecture, whereas ND group showed congestion in blood vessels and sinusoids, cytoplasmic changes, pyknotic nucleus, inflammatory cells, karyolysis, necrosis, and massive damage of hepatic tissue. ND+WS100 group showed few necrotic cells and pyknotic nucleus compared to ND group. ND+WS200 group showed mild hepatic sinusoidal congestion with few pyknotic cells. ND+WS400 group also showed mild hepatic sinusoidal congestion with almost normal cellular architecture similar to control group [Figure 3].

Kidney of control group rats showed parenchyma with well-defined glomerulus and tubules. ND group showed marked congestion in capillaries and atrophy of glomerulus, focal cytoplasmic congestion, and degenerative changes in the proximal convoluted tubules. ND+WS100 showed mild degenerative changes in glomerulus with normal renal architecture, ND+WS200 group showed mild congestion, and ND+WS 400 group also showed mild cytoplasmic changes with normal renal architecture [Figure 4].

DISCUSSION

The side effects caused by AAS such as ND are shown in many convincing works.[24,25] Evidence supports that ND could easily interrupt the redox homeostasis of the liver, heart, and kidney tissues of Wistar rats.[26] To date, there are no convincing therapeutic agents that would ameliorate the ND-induced complications, and hence, the present work tested the efficacy of WS in ND-induced complications using rat model. The key findings revealed elevation of serum levels of liver marker enzymes, namely, ALT, AST, ALP, as well as the serum levels of TP, creatinine, and urea in ND group as compared with control. Regarding the body weight, the ND group showed a marginal reduction in body weight compared to control group and such reductions in body weight may be related with the reduction in food ingestion, since the rats treated with different doses of ND (5, 15, and 45 mg/kg) presented lower weight gain.[27,28] The present findings are in agreement with the earlier works.[29] that showed increased serum levels of the AST and ALT after ND administration to rats. The serum level changes discussed above are indications of abrupt changes in the liver and kidney functions.[30,31] WS drug treatment (WS100, WS200, and WS400 mg/kg) to ND-induced rats significantly reversed the marker enzymes and the biochemical parameters to almost near-normal levels.

It was shown that elevated activities of cytochrome p-450 in the liver and kidney tissues of ND-induced mice could be indications for the tissue level alterations caused by anabolic steroids.[32] In the present work, histopathology data revealed severe alterations in the architecture

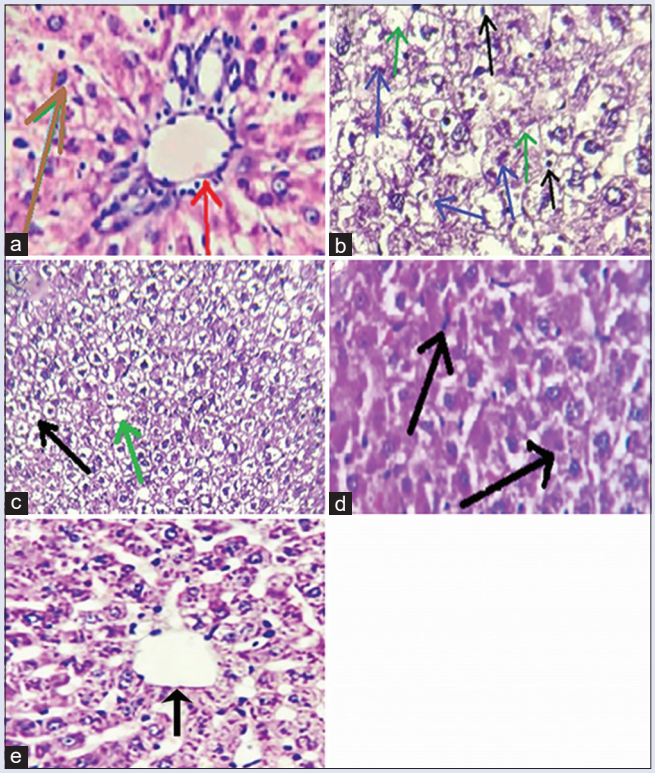

Figure 3: Representative images of hepatic tissue stained with H and E (×40). (a) Control group showed normal hepatic architecture. (b) nandrolone decanoate group showed congestion in blood vessels and sinusoids, necrosis (green arrow), karyolysis (blue arrow), pyknotic nucleus (black arrow), (c) nandrolone decanoate+*Withania somnifera* 100 group showed mild congestion with few pyknotic nucleus (black arrow) and necrosis (green arrow), (d) nandrolone decanoate+*Withania somnifera* 200 group showed mild congestion with few pyknotic nucleus (black arrow), (e) nandrolone decanoate+*Withania somnifera* 400 group showed mild cytoplasmic changes with normal hepatic architecture. Central vein-black arrow

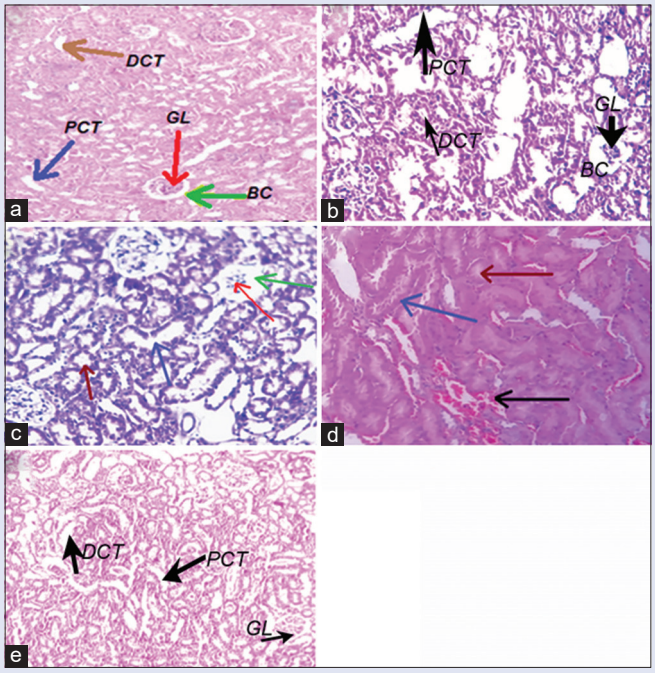

Figure 4: Representative images of kidney tissue stained with H and E (×40). (a) Control group showed normal renal architecture (b) nandrolone decanoate group showed marked congestion in capillaries, atrophy of glomerulus (black arrow), PCT degenerative changes (c) nandrolone decanoate+*Withania somnifera* 100 showed mild degenerative changes of glomerulus (d) nandrolone decanoate+*Withania somnifera* 200 group showed mild congestion (e) nandrolone decanoate+*Withania somnifera* 400 group showed mild cytoplasmic changes with normal renal architecture. GL: Glomerulus, BC: Bowman's capsule, PCT: Proximal convoluted tubule, DCT: Distal convoluted tubule

of liver and kidney of ND-induced rats. The histopathological assessment clearly indicates the cellular damages such as necrosis, karyolysis, congestion of blood vessels and sinusoids in ND group[33] and degenerative changes in kidney,[34,35] and such cellular damages may be due to decreased total antioxidant capacity.[36] Moreover, it was reported that the thickness of renal parenchyma and renal volume were drastically increased in bodybuilders after anabolic steroid usage implicating kidney dysfunctions.[37] After treatment with WS, the number of necrotic cells, nuclear and cytoplasmic changes, as well as congestion was significantly reduced in ND+WS100, ND+WS200, and ND+WS400 groups. The root powder of WS has various bioactive ingredients including bioflavonoids with potential antioxidant functions that might have rendered cellular protection from oxidative stress and free radical-mediated complications.[38] WS root showed the potential to reverse the liver and kidney tissue damage caused by cadmium toxicity, evidenced through assessment of liver enzyme (ALT) and kidney (creatinine and blood urea) markers, and those findings are indicative of hepatorenal protection offered by WS root against ND toxicity.[39]

Withanolide-rich fractions extracted from WS roots have also rendered hepatoprotective functions through anti-inflammatory and antioxidative stress mechanisms by inhibition of TNF-α, IL-1β, and iNOS expression levels.[16] Nrf2-dependent protection against liver injury was also seen in withanolides isolated from WS.[40] It is also evident that oral administration of WS (250 and 500 mg/kg) reduced the nephrotoxicity and protected the kidneys from bromobenzene-induced renal damage and mitochondrial dysfunction in rats.[41] The same group had shown that the elevated kidney function markers such as urea and creatinine were controlled after WS drug treatment.

Other works support the nephroprotective functions of WS in gentamicin-/cisplatin-induced kidney injury in rats that could be attributed due to its enhanced antioxidant capacity and anti-inflammatory functions.[42,43] The present work seeks insight for the first time into the hepatoprotective and nephroprotective functions of WS extract in ND-induced complications using rat model. Hence, WS holds potential as a phytotherapeutic agent to reduce the complications and side effects caused by anabolic steroids.

CONCLUSION

The present study findings indicate that water emulsion of WS root powder has shown a considerable protective effect against ND-induced complications in Wistar rats through evaluation of serum biochemical and tissue histopathological alterations. Further detailed investigations are, however, warranted to explore in-depth, the efficacy of WS drug against ND-induced complications at the molecular level. Therefore, our preliminary research findings are interesting, first of its kind, and thereby suggest that WS drug could be a viable and promising agent to overcome the complications associated with anabolic steroid usage.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. van Amsterdam J, Opperhuizen A, Hartgens F. Adverse health effects of anabolic-androgenic steroids. Regul Toxicol Pharmacol 2010;57:117-23.
2. Shahidi NT. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clin Ther 2001;23:1355-90.
3. Yesalis CE, Bahrke MS. Anabolic-androgenic steroids. Current issues. Sports Med 1995;19:326-40.
4. Tamaki T, Shiraishi T, Takeda H, Matsumiya T, Roy RR, Edgerton VR, *et al.* Nandrolone decanoate enhances hypothalamic biogenic amines in rats. Med Sci Sports Exerc 2003;35:32-8.
5. Belardin LB, Simão VA, Leite GA, Chuffa LG, Camargo IC. Dose-dependent effects and reversibility of the injuries caused by nandrolone decanoate in uterine tissue and fertility of rats. Birth Defects Res B Dev Reprod Toxicol 2014;101:168-77.
6. Boada LD, Zumbado M, Torres S, López A, Díaz-Chico BN, Cabrera JJ, *et al.* Evaluation of acute and chronic hepatotoxic effects exerted by anabolic-androgenic steroid stanozolol in adult male rats. Arch Toxicol 1999;73:465-72.
7. Kopera H. The history of anabolic steroids and a review of clinical experience with anabolic steroids. Acta Endocrinol Suppl (Copenh) 1985;271:11-8.
8. Frankenfeld SP, Oliveira LP, Ortenzi VH, Rego-Monteiro IC, Chaves EA, Ferreira AC, *et al.* The anabolic androgenic steroid nandrolone decanoate disrupts redox homeostasis in liver, heart and kidney of male wistar rats. PLoS One 2014;9:e102699.
9. Samieinasab MR, Shahraki MR, Samieinasab F, Najafi S. Influence of nandrolone decanoate administration on serum lipids and liver enzymes in rats. ARYA Atheroscler 2015;11:256-60.
10. Kulkarni SK, Verma AV. Ashwagandha and Brahmi nootropic and deaddiction profile of psychotropic indigenousplants. Drugs Today 1993;29:257-63.
11. Kulkarni SK, Dhir A. *Withania somnifera*: An Indian ginseng. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:1093-105.
12. Kumar Paul R. *In vitro* Antioxidant Activity of *Withania somnifera* Root. Int J Adv Res Chem Sci 2016;3:45-56.
13. Prince Sabina E, Rasool M, Vedi M, Navaneethan D, Ravichander M, Parthasarthy P, *et al.* Hepatoprotective and antioxidant potential of *Withania somnifera* against paracetamol-induced liver damage in rats. Int J Pharm Pharm Sci 2013;5:975-1491.
14. Akbarsha MA, Vijendrakumar S, Kadalmani B, Girija R, Faridha A. Curative property of *Withania somnifera* Dunal root in the context of carbendazim-induced histopathological changes in the liver and kidney of rat. Phytomedicine 2000;7:499-507.
15. Jeyanthi T, Subramanian P. Protective effect of *Withania somnifera* root powder on lipid peroxidation and antioxidant status in gentamicin-induced nephrotoxic rats. J Basic Clin Physiol Pharmacol 2010;21:61-78.
16. Devkar ST, Kandhare AD, Zanwar AA, Jagtap SD, Katyare SS, Bodhankar SL, *et al.* Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: Decisive role of TNF-α, IL-1β, COX-II and iNOS. Pharm Biol 2016;54:2394-403.
17. Bharavi K, Reddy AG, Rao GS, Reddy AR, Rao SV. Reversal of cadmium-induced oxidative stress in chicken by herbal adaptogens *Withania somnifera* and *Ocimum sanctum*. Toxicol Int 2010;17:59-63.
18. Chaurasia SS, Panda S, Kar A. *Withania somnifera* root extract in the regulation of lead-induced oxidative damage in male mouse. Pharmacol Res 2000;41:663-6.
19. Pandey A, Bani S, Dutt P, Kumar Satti N, Avtar Suri K, Nabi Qazi G, *et al.* Multifunctional neuroprotective effect of withanone, a compound from *Withania somnifera* roots in alleviating cognitive dysfunction. Cytokine 2018;102:211-21.
20. Sarbishegi M, Heidari Z, Mahmoudzadeh-Sagheb H, Valizadeh M, Doostkami M. Neuroprotective effects of *Withania coagulans* root extract on CA1 hippocampus following cerebral ischemia in rats. Avicenna J Phytomed 2016;6:399-409.
21. Chandran U, Patwardhan B. Network ethnopharmacological evaluation of the immunomodulatory activity of *Withania somnifera*. J Ethnopharmacol 2017;197:250-6.
22. Kaur T, Singh H, Mishra R, Manchanda S, Gupta M, Saini V, *et al. Withania somnifera* as a potential anxiolytic and immunomodulatory agent in acute sleep deprived female wistar rats. Mol Cell Biochem 2017;427:91-101.
23. Rasool M, Varalakshmi P. Immunomodulatory role of *Withania somnifera* root powder on experimental induced inflammation: An *in vivo* and *in vitro* study. Vascul Pharmacol 2006;44:406-10.
24. Almaiman AA, Almaiman SH, Elagamy EI, Al Wutayd O, Almarzuqi M, Alzunaidi R, *et al.* Side effects of anabolic steroids used by athletes at unaizah gyms, Saudi Arabia: A pilot study. J Sports Med Phys Fitness 2019;59:489-95.
25. Pozzi R, Fernandes KR, de Moura CF, Ferrari RA, Fernandes KP, Renno AC, *et al.* Nandrolone decanoate induces genetic damage in multiple organs of rats. Arch Environ Contam Toxicol 2013;64:514-8.
26. Frankenfeld SP, de Oliveira LP, Ignacio DL, Coelho RG, Mattos MN, Ferreira AC, *et al.* Nandrolone decanoate inhibits gluconeogenesis and decreases fasting glucose in wistar male rats. J Endocrinol 2014;220:143-53.
27. Rezende Soares MC, de Abreu IC, Assenço F, da Rocha Borges MO. Nandrolone decanoate increases the left ventricular wall but attenuates the cavity increase caused by swimming training in rats. Rev Bras Med Esporte 2011;17:420-4.
28. Nahrendorf M, Frantz S, Hu K, von zur Mühlen C, Tomaszewski M, Scheuermann H, *et al.* Effect of testosterone on post-myocardial infarction remodeling and function. Cardiovasc Res 2003;57:370-8.
29. Vieira RP, França RF, Damaceno-Rodrigues NR, Dolhnikoff M, Caldini EG, Carvalho CR, *et al.* Dose-dependent hepatic response to subchronic administration of nandrolone decanoate. Med Sci Sports Exerc 2008;40:842-7.
30. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC; Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008;47:1363-70.
31. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 2000;342:1266-71.
32. Acharjee BK, Mahanta R. Enhanced hepatic and kidney cytochrome p-450 activities in nandrolone decanoate treated albino mice. Drug Metab Lett 2009;3:120-4.
33. Saad Al-Dhuayan I. Possible protective role of whey protein on the rat's liver tissues treated with nandrolone decanoate. Pak J Biol Sci 2018;21:262-74.
34. Kahal A, Allem R. Reversible effects of anabolic steroid abuse on cyto-architectures of the heart, kidneys and testis in adult male mice. Biomed Pharmacother 2018;106:917-22.
35. Shalaby AM, El Shaer DF. Lycopene protects against renal cortical damage induced by nandrolone decanoate in adult male rats. Ann Anat 2019;224:142-52.
36. Joukar S, Vahidi R, Farsinejad A, Asadi-Shekaari M, Shahouzehi B. Ameliorative effects of endurance exercise with two different intensities on nandrolone decanoate-induced neurodegeneration in rats: Involving redox and apoptotic systems. Neurotox Res 2017;32:41-9.
37. Kantarci UH, Punduk Z, Senarslan O, Dirik A. Evaluation of anabolic steroid induced renal damage with sonography in bodybuilders. J Sports Med Phys Fitness 2018;58:1681-7.
38. Jeyanthi T, Subramanian P. Nephroprotective effect of *Withania somnifera*: A dose-dependent study. Ren Fail 2009;31:814-21.
39. Bharavi K, Reddy AG, Rao GS, Kumar PR, Kumar DS, Prasadini PP, *et al.* Prevention of cadmium bioaccumulation by herbal adaptogens. Indian J Pharmacol 2011;43:45-9.
40. Palliyaguru DL, Chartoumpekis DV, Wakabayashi N, Skoko JJ, Yagishita Y, Singh SV, *et al.* Withaferin A induces Nrf2-dependent protection against liver injury: Role of keap1-independent mechanisms. Free Radic Biol Med 2016;101:116-28.
41. Vedi M, Rasool M, Sabina EP. Protective effect of administration of *Withania somifera* against bromobenzene induced nephrotoxicity and mitochondrial oxidative stress in rats. Ren Fail 2014;36:1095-103.
42. Govindappa P, Gautam V, Tripathi SM, Sahni YP, Raghavendra HL. Effect of Withania somnifera on gentamicin induced renal lesions in rats. Rev Bras Farmacogn 2019;29:234-40.
43. Sharma S, Joshi A, Hemalatha S. Protective effect of *Withania coagulans* fruit extract on cisplatin-induced nephrotoxicity in rats. Pharmacognosy Res 2017;9:354-61.