

The protective effect of *Withania somnifera* against oxidative damage caused by ethanol in the testes of adult male rats

Divya Bhargavan^{1*}, B. Deepa¹, Harish Shetty², A. P. Krishna³

¹Department of Pharmacology, K. S. Hegde Medical Academy, Mangalore, Karnataka, India, ²Department of OBG, K. S. Hegde Medical Academy, Mangalore, Karnataka, India, ³Department of Physiology, K. S. Hegde Medical Academy, Mangalore, Karnataka, India

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***Correspondence to:**

Divya Bhargavan,
Email: divyabhargavan@rediffmail.com

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ABSTRACT

Background: To investigate the effect of *Withania somnifera* (WS) in preventing the damage caused by alcohol on testis.

Methods: Adult male Wistar rats were divided into 4 groups. Group A - control, Group B - WS 200 mg orally, Group C - alcohol 4 g/kg orally, and Group D - WS + alcohol. The sperm parameters were examined. Testicular tissues were examined for biochemical (glutathione [GSH] peroxidase, superoxide dismutase, catalase (CAT), malondialdehyde [MDA]) analysis, and histopathological changes.

Results: Biochemical parameters revealed tissue oxidative stress in alcohol group which was evidenced as increase in MDA level and reduction in CAT and GSH activities in testes which was reduced in co-treatment group. WS significantly reduced alcohol-induced sperm shape abnormality and sperm count. The alcohol-induced changes in histopathologic findings were partially reversed by treatment with WS.

Conclusion: These observations suggest that the antioxidant property of WS might have contributed for its ability to ameliorate the testicular toxicity caused by alcohol.

Keywords: Alcohol, Oxidative stress, Testicular toxicity, *Withania somnifera*

INTRODUCTION

The deleterious effects of alcohol on human body are well-documented, as it can adversely affect many organs such as liver, lungs, and testis. Alcohol is a reproductive toxin. Alcohol abuse is known to impair testosterone production and testicular atrophy in men. Those changes can result in impotence, infertility, and reduced male secondary sexual characteristics.¹

Chronic alcoholics are found to have sperm abnormalities with low sperm count, impaired sperm motility and structural abnormality.² Alcohol is also known to impair reproductive performance in experimental animals and human.³ Alcohol has shown to cause an adverse effect on sperm maturation,

which may be affected due to decrease in testosterone and dihydrotestosterone level and the epididymal secretory products.⁴

Herbal products are gaining popularity especially in the treatment of male sexual disorders, notable among these is *Withania somnifera* (WS). WS is widely used in folk medicine to improve sperm count and semen quality.⁵ WS also known as Ashwagandha, Indian ginseng, and winter cherry is an important medicinal plant, widely used as a home remedy for several diseases in India as well as other parts of the world. WS is a small, woody shrub in the Solanaceae family that grows about two feet in height, found throughout the drier parts of India in waste places and on bunds.⁶

Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins, and senile dementia.⁷ Clinical trials and animal research support the use of WS for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease.⁹

However reputed for its antioxidant potential, there is little data on the possible palliative effect of WS in alcohol-induced testicular injury. The current study was designed to investigate the effect of WS in reducing the oxidative stress caused by alcohol on rat testis.

METHODS

Preparation of WS ethanolic root extract (WSEE)

WS roots were purchased from the local market authenticated by a reliable source. WS roots were cut into 2 cm pieces and shade dried. They were coarsely powdered. The powder was subjected to soxhlet extraction with 95% ethyl alcohol for 72 hrs at temperature of 70-80°C. The resulting extract was concentrated, and the solvent was evaporated using rotary evaporator and stored at 4°C for future use. This was then dissolved in distilled water and administered orally to the rats.

Animals

The experiments were conducted in the animal house of K S Hegde Medical academy. The study protocol was approved by the Animal Ethical Committee, KSHEMA and the experiments on animals were performed in accordance to the CPCSEA guidelines.

Healthy adult male albino rats were procured from the institutional animal house, KSHEMA. The animals were housed in polypropylene cages with adequate bedding and maintained in a well-ventilated room in 12 hrs light and dark cycle with free access to food pellets and drinking water.

Study design

The animals were randomly segregated into four groups (n=6).

Group A: Distilled water and served as control

Group B: WSEE 200 mg/kg

Group C: Alcohol 4 g/kg

Group D: WSEE 200 mg/kg and alcohol 4 g/kg after 4 hrs.

All the animals in groups received alcohol and WSEE orally for 28 days, which is the time taken to complete a spermatogenic cycle in rats.¹⁰ Absolute alcohol was 50% diluted before administering, and the dose of alcohol was selected based on previous study.¹¹

Sample collection

After the experimental period, rats were weighed and anesthetized with ketamine 150 mg/kg body weight. The testes and epididymis were dissected out soon after sacrifice, cleared of fat and washed with saline, soaked on blotting paper and weights were recorded.

One of the testes was used for histological evaluation, and the other testis was used for the biochemical assays.

Determination of sperm characteristics

The caudal epididymis was minced in 1 ml of pre-warmed phosphate buffer saline, incubated at 37°C for 10 mins and filtering through a mesh. One drop of sperm suspension was placed on a glass slide, covered with a coverslip. The motility of the epididymal sperm was evaluated microscopically within 2-3 mins of their isolation from the epididymis and data were expressed as percentage of motile sperm of the total sperm counted.¹²

Sperm count was done under a microscope with the aid of the Neubauer hemocytometer.

The sperm suspension was mixed with equal volume of 1% eosin - Y and smears were prepared on clean glass slides, slides were viewed by bright-field microscope with magnification of $\times 400$.¹³ About 200 sperms was examined to determine the morphological abnormalities which were classified as amorphous, hookless, coiled, tailless, and finally represented as percentage total abnormality.¹⁴

Determination of enzyme activities

The testis and epididymis was washed with ice cold physiological saline, and 0.5 g of the tissue was homogenized in 0.1 M Tris-HCl buffer of pH 7.5 and centrifuged in a refrigerated centrifuge at 10,000 g for 30 mins. The supernatant was used as enzyme source for various assays.

The total protein was estimated by biuret method. The estimation of superoxide dismutase was carried out by Beauchamp and Fridovich method.^{15,16} The catalase (CAT) activity was determined at 560 nm the consumption of exogenous H_2O_2 measured according to the modified method of Aebi.¹⁷ Glutathione (GSH) was assayed according to the method described by Ellman¹⁸ malondialdehyde (MDA) was assayed according to method described by Esterbauer and Cheeseman.¹⁹

Intratesticular testosterone was measured using LDN kit by enzyme-linked immunosorbent assay method according to manufacturer's protocol.

Tissue preparation for histological analysis

The testis was fixed in formalin for 24 hrs and then dehydrated by passing through ascending grades of

alcohol (70%, 80%, 90%, and absolute alcohol). After dehydration, tissues were cleared in xylene, infiltrated, and then embedded in paraffin wax. Each testis was sectioned along the long axis in 5 mm thickness with the aid of a microtome. The deparaffinized sections were stained with hematoxylin and eosin according to routine procedures for light microscopy. The changes observed were recorded and photomicrographs of the most prominent pathological lesions taken.²⁰

Statistical analysis

The data were analyzed by means of Kruskal–Wallis test followed by Mann–Whitney U-test for intercomparisons using SPSS package for windows 11. The $p < 0.05$ was considered to be statistically significant.

RESULTS

The testicular and the body weight were significantly decreased in rats of alcohol toxicity group compared with that of control ($p < 0.01$). Treatment with WSEE significantly prevented the decline of testicular weight and body weight. In alcohol treated group, the sperm quality of rat was severely affected by alcohol seen as a decrease in sperm count, motility and increased sperm abnormality ($p < 0.01$). While alcohol+WSEE co-treatment group significantly increased these variables compared to the alcohol treatment group (Table 1).

In the alcohol toxicity group, MDA levels were increased and the GSH, CAT levels have decreased when compared to control group. Whereas in the alcohol and WSEE co-treatment group the MDA levels were reduced and the antioxidant parameters were raised indicating it can combat the oxidative stress (Table 2).

Microscopical examination of testicular tissues in the control group showed normal architecture of seminiferous tubules, well preserved sertoli cells and well-delineated tubular basement membrane. The testicular interstitium contained leydig cells and macrophages without any inflammatory cell infiltrates and histopathological lesions.

However, alcohol-induced changes include irregular seminiferous tubules with epithelial sloughing and cellular degeneration. Germ cells showed disorganized maturation, were degraded and not attached to the epithelium. Interstitium shows edema and vacuolization and presence of multinucleated giant cells.

With alcohol and WSEE co-administration, testicular architecture is maintained with tubules showing epithelial sloughing and at areas appears denuded. The number of sperm cells in the lumina was better than the alcohol group. Germ cells show disorganized maturation. Interstitium appears edematous, but there is no vacuolization. The pathologic changes in seminiferous tubules have recovered (Figure 1).

DISCUSSION

Chronic alcohol consumption has a detrimental effect on male reproductive hormones and on semen quality.²¹ It is

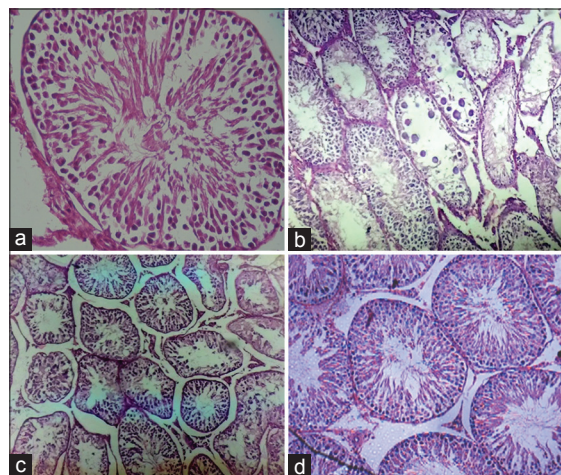


Figure 1: Histological testis sections (H and E) of, (a) control, normal arrangement of seminiferous tubules, sperms and interstitial cells $\times 40$, (b and c) alcohol group showing disorganization of spermatogenic cells, degeneration of germinal epithelium, and multinucleated cells $\times 10$, (d) *Withania somnifera* ethanolic root extract + Alcohol co-treatment group, near normal tubules, $\times 10$.

Table 1: Effects of WSEE on sperm quality, testis and epididymis weight in alcohol treated rats.

Parameters	Control	Alcohol	WSEE	Alcohol+WSEE
Final body weight (g)	226.53 \pm 23.4	184.6 \pm 18.9*	260.6 \pm 36.4	209.43 \pm 22.4
Testes weight (g)	3.15 \pm 0.78	2.71 \pm 0.11*	3.2 \pm 0.81	3.09 \pm 0.19 [‡]
Epididymis weight (g)	1.14 \pm 0.21	1 \pm 0.29	1.2 \pm 0.125	1.12 \pm 0.29
Sperm count ($\times 10^6$)	54 \pm 9	37.2 \pm 17.25*	62.5 \pm 8.5 [‡]	51.5 \pm 17.75
Sperm motility %	78 \pm 5.24	61 \pm 4.3*	81.5 \pm 3.25	72.5 \pm 3.83 [‡]
Sperm abnormality %	20 \pm 1.41	39 \pm 4.5*	15.5 \pm 1.8*	30.6 \pm 4.6**

Data is expressed as mean \pm standard deviation. * $p < 0.01$ compared with control, [‡] $p < 0.05$ compared with control, [‡] $p < 0.01$ compared with alcohol, ^{**} $p < 0.05$ compared with alcohol, The data were analyzed by means of Kruskal–Wallis test followed by Mann–Whitney U-test for intercomparisons. WSEE: *Withania somnifera* ethanolic root extract

Table 2: Effect of WSEE on biochemical parameters in alcohol treated rats.

Parameters	Control	Alcohol	WSEE	Alcohol+WSEE
Protein mg/g tissue	96.93±13.6	40.47±19.79*	99.7±7.5	89.83±8.03 [‡]
GSH µg/g tissue	194.74±80.22	101.8±48.18*	170.65±56.3 [‡]	151.57±82.43 [‡]
SOD U/g tissue	151.46±55.13	222±131.9	151.46±55.13	176.29±34.59
CAT mmoles/ml	1.9277±0.17	0.796±0.153 *	1.88±0.257	1.45±0.312* [‡]
MDA mmoles/g	134.09±48.64	237.82±83.61*	125.54±54.1	166.87±17.81*
Testosterone ng/ml	15.76±4.2	9.84±3.2*	16.3±4.2	13.3±2.4

Data is expressed as median±interquartile range. *p<0.01 compared with control, [‡]p<0.05 compared with control, [‡]p<0.01 compared with alcohol, [‡]p<0.05 compared with alcohol. The data were analyzed by means of Kruskal–Wallis test followed by Mann–Whitney U-test for intercomparisons. WSEE: *Withania somnifera* ethanolic root extract, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde

postulated that free radicals and lipid peroxidation play an important role in the testicular toxicity by alcohol. Testis has an abundance of highly unsaturated fatty acid and the presence of potential reactive oxygen species generating system, and hence highly vulnerable to oxidative stress. Lipid peroxidation is the major end-point of oxidative damage resulting from chronic ethanol administration.²²

MDA was found significantly increased in alcohol group, whereas there was a prominent decrease of antioxidant system in the testis. It is postulated that ethanol inhibits GSH synthesis and deplete GSH levels in tissues and generally impair the antioxidative defense mechanism in human and experimental animals.²³ The increase in MDA levels and decrease in CAT and GSH activities could reflect as an adverse effect on the antioxidant system in rat testis.

Furthermore, loss of body weight and testicular weight, decrease in sperm count, motility, and increase in abnormality and decrease in testosterone level, reveals the toxic nature of alcohol on testis.

The decrease in testosterone with alcohol is consistent with other studies and may be due to the deleterious effect on testosterone producing leydig cell.³

Alcohol caused histological changes in the seminiferous tubules resulting in suppression of spermatogenesis. Atrophy of seminiferous tubules which presented as loss of spermatogenic cells indicates failure of spermatogenesis which is reflected as decrease in sperm count as points to free radical attack on the testis.

Alcohol also impairs the function of the testicular sertoli cells that play an important role in sperm maturation.¹

Our data confirms and supports other studies that chronic alcohol administration induces oxidative stress.

WS significantly increased sperm count, motility, and reduced sperm abnormality. The semen quality is seen to be improved with WS in alcohol treatment group. WS also decreases MDA level and increases antioxidant activity,

shows activity of WS in combating oxidative stress. The significant increase of testosterone levels in the testes show that WS stimulated the male reproductive function.

There was a significant increase in the body weight and testis weight after treatment of WS. In addition, alcohol induced deterioration of germ cells and other histopathological findings were improved by WS and alcohol co-treatment.

The increase in the body weight of WS treated rats could be due to the androgenic properties of this plant since androgens possess anabolic activity.

There are several studies supporting on the benefits of antioxidants in protecting the testis against oxidative damage.²³⁻²⁵ The antioxidant potential of WS has already been reported. Previous study has reported that it ameliorates against cadmium-induced oxidative damage by antioxidant potential.²⁶

Besides a recent study revealed that WS decreased stress, improved the level of antioxidants and improved overall semen quality in a significant number of individuals.²⁷

CONCLUSION

The finding of our study indicates that WS appears as a promising candidate in preventing the alcohol induced testicular toxicity. This protective effect of WS is attributed to its inhibition of oxidative stress and scavenging of free radicals.

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