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Original article

In vivo antioxidant efficacy and therapeutic potential of *Artemisia brevifolia* leaves extract against CCl_4-induced reproductive damages in male albino rats

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ABSTRACT

The current study was aimed to investigate the alleviative effect of *Artemisia brevifolia* (*A. brevifolia*) plant extract against CCl_4-induced testicular damage in male albino rats. Forty-eight male albino rats were categorized into eight equal experimental groups i.e., control, vehicle control, CCl_4 (1 ml/kg), CCl_4 + silymarin, CCl_4 + *A. brevifolia* (150 mg/kg), CCl_4 + *A. brevifolia* (300 mg/kg), *A. brevifolia* (150 mg/kg) and *A. brevifolia* (300 mg/kg). After 56 days, blood and testicular samples were collected, and antioxidant enzyme activity, lipid peroxidation, hormonal concentration, daily sperm production (DSP), and histomorphometry were analyzed. The animals treated with the *A. brevifolia* extract exhibited a significant ($p < 0.05$) increase in the catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR) activity, and significant ($p < 0.05$) decrease in thiobarbituric acid reactive substances (TBARS) level, potentially damaged by CCl_4. Significant ($p < 0.05$) restoration in luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone concentrations, and DSP was observed in the *A. brevifolia* treated groups. Besides, CCl_4 significantly ($p < 0.05$) dysregulated the lipid profile by increasing cholesterol, LDL, and triglycerides, while reducing HDL. The histopathological analysis evinced that CCl_4 significantly ($p < 0.05$) damaged the testicular tissues. However, *A. brevifolia* treatment considerably abated the detrimental effects of CCl_4 in rat testes. In conclusion, our results suggested the therapeutic role of *A. brevifolia* in oxidative stress-related disorders of testes.
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1. Introduction

Spermatogenesis in animals is severely affected by exposure to various environmental toxicants, causing abnormal sperm morphology, low sperm quality, and oligospermia (Aitken et al., 2004; Sharpe, 2010). These environmental toxicants mostly entered the environment due to industrial waste. After becoming part of the environment, such toxicants enter the terrestrial animals through contaminated air, foodstuff, and water (Aitken et al., 2004; Wirth and Mijal, 2010). These toxicants affect the endocrine system, which may cause irregularities in male reproductive systems (Benoff et al., 2009; Coutts et al., 2007).

Carbon tetrachloride (CCl_4) is extensively used as a standard toxicant to stimulate chemical toxicity in testes (Samad et al., 2020). Cytochrome P450 enzymes are present in testes, which convert the CCl_4 into a potentially toxic substance (Naz et al., 2014). Initially, CCl_4 damages the tissues by producing trichloromethyl and trichloromethyl peroxyl radicals with the help of cytochrome P450 enzymes (Halliwell and Gutteridge, 2007). When trichloro-

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methyl free radicals reach a very high level, they instigate oxidation in membrane protein, lipids and finally culminate in histopathological deteriorations (Abdel Moneim and El-Khadragy, 2013).

Some medicinal plant extracts contain antioxidants that become incorporated into the body and support the antioxidants by reducing free radicals and increasing the life duration of biological processes (Khan and Younus, 2011; Wang et al., 2019; Ijaz et al., 2020a). Such antioxidant-containing plant extracts have shown therapeutic potential against chemically induced damages (Bakar et al., 2019). Herbal remedies have been witnessed as a miracle for treating numerous diseases (Bari et al., 2020; Zhao et al., 2020). Artemisia is a widely distributed genus of family Asteraceae, comprising 20–500 descriptive species associated with Anthemideae (Kubitzki, 2007; Ling et al., 2006). Central Asia is regarded as the parental area home to many different genus Artemisia varieties, from where its other species originated and spread to the whole world. This encompasses herbs and shrubs, which mostly flourish in sustainable ecosystems. They are primarily used for food and ornamental purposes (Ling et al., 2006). *A. brevifolia* Wall. ex DC. (Asteraceae), locally known as Afsanteen, has been used extensively in ethnoveterinary medicines in Pakistan as an anthelmintic. However, the protective effects of *A. brevifolia* on the male reproductive system have not been investigated yet. Therefore, the current study was performed to evaluate the therapeutic properties of *A. brevifolia* to counter CCl_4-induced testicular injury in male albino rats.

2. Materials and methods

2.1. Collection and preparation of plant extract

A. brevifolia plant was collected from the Noori Top (Naran Vally, Mansehra District, KPK, Pakistan) in September 2018. The leaves of *A. brevifolia* were dehydrated under shade for 15 days and ground into powder. 1 kg of powder was dipped in the crude-methanol for 72 h and repeated process for two times before filtration was completed through Whatman (No. 1) filter and evaporation of methanol was conducted on a rotary evaporator in lower force at 40 °C. We stocked the obtained plant extract at 4 °C for subsequent analysis.

2.2. Experimental protocol

Mature male albino rats (160–180 g) were housed in the UAF Pharmacology department's animal house at 25 ± 2 °C. Rats were provided with tap water and a balanced diet. The study was approved by the ethical committee, University of Agriculture, Faisalabad, Pakistan (DGS No. 6477-80). Forty-eight mature male albino rats were divided into eight experimental groups comprising of 6 rats each. The first group was designated as the control group. The second group was administered with 1 ml/kg DMSO orally (Vehicle control), while the third group was administered with CCl_4 dissolved in olive oil (1 ml/kg). The fourth group was treated with a 100 mg/kg dose of silymarin with CCl_4, where the Fifth and sixth groups were served with the extract of *A. brevifolia* (150 mg/kg and 300 mg/kg) + CCl_4 in order. However, groups VII and VIII were treated only with *A. brevifolia* extracts at the rate of 150 and 300 mg/kg, respectively. The dose of CCl_4 was used following a previous study by Samad et al. (2019), while quantities of *A. brevifolia* were selected following the dose used by Ahsan et al. (2020). On the 56th day of trial, rats were given anesthesia by diethyl ether and then decapitated. Retro-orbital venous plexus was used for blood collection with the help of a fine needle, and serum was separated from the blood. On the 56th day of trial, rats were decapitated after anesthetization. Blood samples were collected in sterilized tubes, stored at 4 °C after centrifugation. Testes were removed, rinsed with the normal saline, homogenized by adding distilled water, and further centrifuged for approximately 15 min at 3000 rpm and stored at −20 °C.

2.3. Biochemical study

Chance and Maehly (1995) procedure was followed to estimate the peroxidase (POD) and catalase (CAT) activity. The method of Nishikimi et al. (1972) was pursued to determine the activity of superoxide dismutase (SOD). The action of glutathione reductase (GR) was assessed by the process of Factor et al. (1998). To carry out the Thiobarbituric acid reactive substances (TBARS), a modified scheme by Iqbal et al. (1996) was trailed.

2.4. Estimation of FSH, LH and testosterone concentrations

Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) concentrations were estimated by using specific kits from Bio-Vendor (Gunma, Japan). ELISA kits (Catalog# EK-311-15; Sensitivity 98%) were used to evaluate the testosterone hormone concentration from serum samples.

2.5. Daily sperm production

Daily sperm production was estimated using the method of Robb et al. (1978). The number of spermatids that were resilient to homogenization was divided by 6.3 for the calculation of DSP.

DSP = Y/6.3Where Y = number of spermatids present in homogenate6.3 = Entire days through spermatids remained in the seminiferous tubules epithelial part.

2.6. Analysis of lipid profile

The evaluation of cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) was performed on blood plasma using commercially available kits (Diagnostic Product Corporation, Los Angeles, USA).

2.7. Histopathological examination

Fixation of the testicular tissues was accomplished in 10% formalin buffer, desiccated in alcohol, and fixed in paraffin wax blocks. Thin sections of 4–5 μm were sliced and fixed on a glass slide, stained with hematoxylin/eosin, and further studied under the light microscope (Nikon Labophot, Japan) at 400×.

2.8. Statistical analysis

The obtained dataset was checked for normality before subjecting it to further data analysis. The final data was presented as Means ± SEM. For comparing various groups, we applied the one-way analysis of variance (ANOVA) followed by Tukey's test with the help of Minitab software. The level of significance was set at $p < 0.05$ during all analyses.

3. Results

3.1. Effect of A. brevifolia on the antioxidant-enzymes and lipid peroxidation

One of the leading objectives was to evaluate the antioxidant enzyme activities such as CAT, SOD, POD, and GR. These enzyme activities showed a significant reduction ($p < 0.05$) in the CCl_4-

administered group than in the control group. Treatment of *A. brevifolia* against CCl_4 indicated significantly improved enzyme activities compared with the CCl_4 treated group. Rats co-treated with *A. brevifolia* and CCl_4 revealed a significant increase in SOD, POD, CAT, and GR activities compared to the CCl_4 group (Table 1). The level of TBARS was substantially ($p < 0.05$) increased in the CCl4 administered group compared to the control group. There was a substantial ($p < 0.05$) reduction in TBARS level was witnessed in CCl_4 + *A. brevifolia* treated rats than the CCl_4 administered group. The *A. brevifolia* significantly decreased TBARS in the CCl_4 treated group, with no remarkable difference from the control group.

3.2. Effect of A. brevifolia on hormonal concentrations and DSP

FSH, LH, and testosterone concentrations were significantly ($p < 0.05$) decreased in the CCl_4-treated group than in the control group. However, in the co-treated groups and *A. brevifolia* alone administered groups, the hormonal level was significantly ($p < 0.05$) improved in a dose-dependent fashion (Table 2). The CCl_4 administered group showed a substantial ($p < 0.05$) reduction in DSP compared to the control group. However, the DSP illustrated similar trends in the control and vehicle control groups. Nonetheless, the DSP displayed restoration in the sperm counts in CCl_4 + *A. brevifolia* treated group. Furthermore, *A. brevifolia* only treated groups showed normal level of DSP.

3.3. Effect of A. brevifolia on lipid profile

The CCl_4 exposure triggered a significant ($p < 0.05$) increase in the triglycerides and LDL levels as compared to the control group albino rats (Table 3). Similarly, we observed a significant ($p < 0.05$) rise in cholesterol level in the CCl_4-intoxicated rats versus control rats. On the contrary, the HDL level displayed an opposite trend and exhibited a significant ($p < 0.05$) decrease in the CCl_4-administered groups versus the control group. The rats treated with *A. brevifolia* and CCl_4 (cotreated) showed a noticeable reduction in triglycerides, cholesterol and LDL, and an increase in HDL level than the CCl_4 treated group.

3.4. Effect of A. brevifolia on histomorphometry of the testicular tissues

We observed a significant ($p < 0.05$) decline in the numbers of spermatogonia, primary and secondary spermatocytes, and spermatids recorded in the CCl_4-treated group as compared to the control group (Table 3). On the other hand, the spermatogonia, primary and secondary spermatocytes, and spermatids displayed

Table 1
Effect of *A. brevifolia* on CAT, SOD, POD, GR and lipid peroxidation (TBARS).

Groups	CAT (U/mg protein)	SOD (U/mg protein)	POD (nmole/mg protein	GR (nM NADPH oxidized/ min/mg tissue)	TBARS (nM/mg tissue)
Control	8.80 ± 0.19^{a}	5.25 ± 0.10^{a}	6.94 ± 0.11^{a}	134.0 ± 3.79^{a}	13.07 ± 0.13^{a}
Vehicle control	8.39 ± 0.03^{a}	5.07 ± 0.05^{a}	6.78 ± 0.05^{a}	124.3 ± 2.96^{a}	13.86 ± 0.10^{a}
CCl_4	3.93 ± 0.13^{d}	1.83 ± 0.08^{c}	2.97 ± 0.06^{d}	59.33 ± 3.38^{d}	25.75 ± 0.18^{d}
CCl_4 + Silymarin	7.88 ± 0.09^{b}	4.84 ± 0.07^{ab}	6.18 ± 0.07^{ab}	114.3 ± 2.96^{b}	15.94 ± 0.15^{a}
CCl_4 + *A. brevifolia* (150 mg/kg)	6.80 ± 0.13^{c}	4.23 ± 0.07^{b}	5.87 ± 0.05^{b}	92.66 ± 2.18^{ab}	18.32 ± 0.40^{b}
CCl_4 + *A. brevifolia* (300 mg/kg)	7.84 ± 0.08^{a}	5.21 ± 0.17^{a}	6.46 ± 0.13^{a}	116.0 ± 2.64^{b}	16.15 ± 0.47^{ab}
A. brevifolia (150 mg/kg)	7.99 ± 0.08^{a}	5.01 ± 0.04^{a}	6.59 ± 0.06^{a}	124.7 ± 1.45^{a}	14.28 ± 0.09^{a}
A. brevifolia (300 mg/kg)	8.35 ± 0.08^{a}	5.06 ± 0.15^{a}	7.04 ± 0.09^{a}	121.0 ± 3.79^{b}	13.97 ± 0.11^{a}

Values having dissimilar superscripts are significantly ($p < 0.05$) different.

Table 2
Effect of *A. brevifolia* on FSH, LH and testosterone concentrations and DSP.

Groups	FSH (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)	DSP $\times 10^6$/gram
Control	3.83 ± 0.08^{a}	2.70 ± 0.04^{a}	4.56 ± 0.05^{a}	22.09 ± 0.29^{a}
Vehicle control	3.74 ± 0.07^{a}	2.57 ± 0.04^{a}	4.47 ± 0.06^{a}	21.00 ± 0.33^{a}
CCl_4	2.01 ± 0.06^{d}	1.04 ± 0.04^{c}	0.96 ± 0.10^{c}	09.62 ± 0.14^{c}
CCl_4 + Silymarin	3.45 ± 0.06^{b}	2.34 ± 0.04^{b}	3.94 ± 0.04^{b}	18.55 ± 0.25^{b}
CCl_4 + *A. brevifolia*(150 mg/kg)	3.24 ± 0.05^{c}	2.37 ± 0.06^{b}	4.04 ± 0.07^{b}	17.33 ± 0.17^{b}
CCl_4 + *A. brevifolia*(300 mg/kg)	3.45 ± 0.03^{b}	2.45 ± 0.06^{bc}	4.39 ± 0.05^{a}	17.89 ± 0.09^{b}
A. brevifolia (150 mg/kg)	3.44 ± 0.05^{b}	2.58 ± 0.02^{a}	4.39 ± 0.02^{a}	18.89 ± 0.09^{b}
A. brevifolia (300 mg/kg)	3.63 ± 0.04^{a}	2.66 ± 0.02^{a}	4.46 ± 0.07^{a}	18.88 ± 0.09^{b}

Values having dissimilar superscripts are significantly ($p < 0.05$) different.

Table 3
Effect of *A. brevifolia* on HDL, LDL, triglycerides and cholesterol.

Groups	HDL (mg dl^{-1})	LDL (mg dl^{-1})	Triglycerides (mg dl^{-1})	Cholesterol (mg dl^{-1})
Control	37.7 ± 1.24^{a}	7.54 ± 0.54^{a}	48.2 ± 2.02^{a}	44.4 ± 1.75^{a}
Vehicle control	36.8 ± 0.78^{a}	7.66 ± 0.71^{a}	49.4 ± 2.46^{a}	45.6 ± 1.41^{a}
CCl_4	13.0 ± 0.94^{d}	19.9 ± 1.08^{c}	89.5 ± 2.94^{c}	66.7 ± 2.22^{c}
CCl_4 + Silymarin	35.3 ± 1.10^{b}	8.33 ± 0.63^{ab}	54.3 ± 2.50^{b}	46.4 ± 1.67^{b}
CCl_4 + *A. brevifolia*(150 mg/kg)	30.5 ± 0.61^{c}	9.72 ± 0.36^{b}	61.0 ± 1.26^{bc}	49.8 ± 1.58^{b}
CCl_4 + *A. brevifolia*(300 mg/kg)	33.8 ± 1.05^{bc}	9.24 ± 0.44^{b}	55.4 ± 2.24^{b}	47.7 ± 1.62^{b}
A. brevifolia (150 mg/kg)	37.5 ± 0.90^{a}	7.68 ± 0.59^{a}	49.9 ± 2.44^{a}	45.7 ± 1.59^{b}
A. brevifolia (300 mg/kg)	38.5 ± 1.06^{a}	7.36 ± 0.51^{a}	47.3 ± 2.51^{a}	44.12 ± 1.57^{b}

Values having dissimilar superscripts are significantly ($p < 0.05$) different.

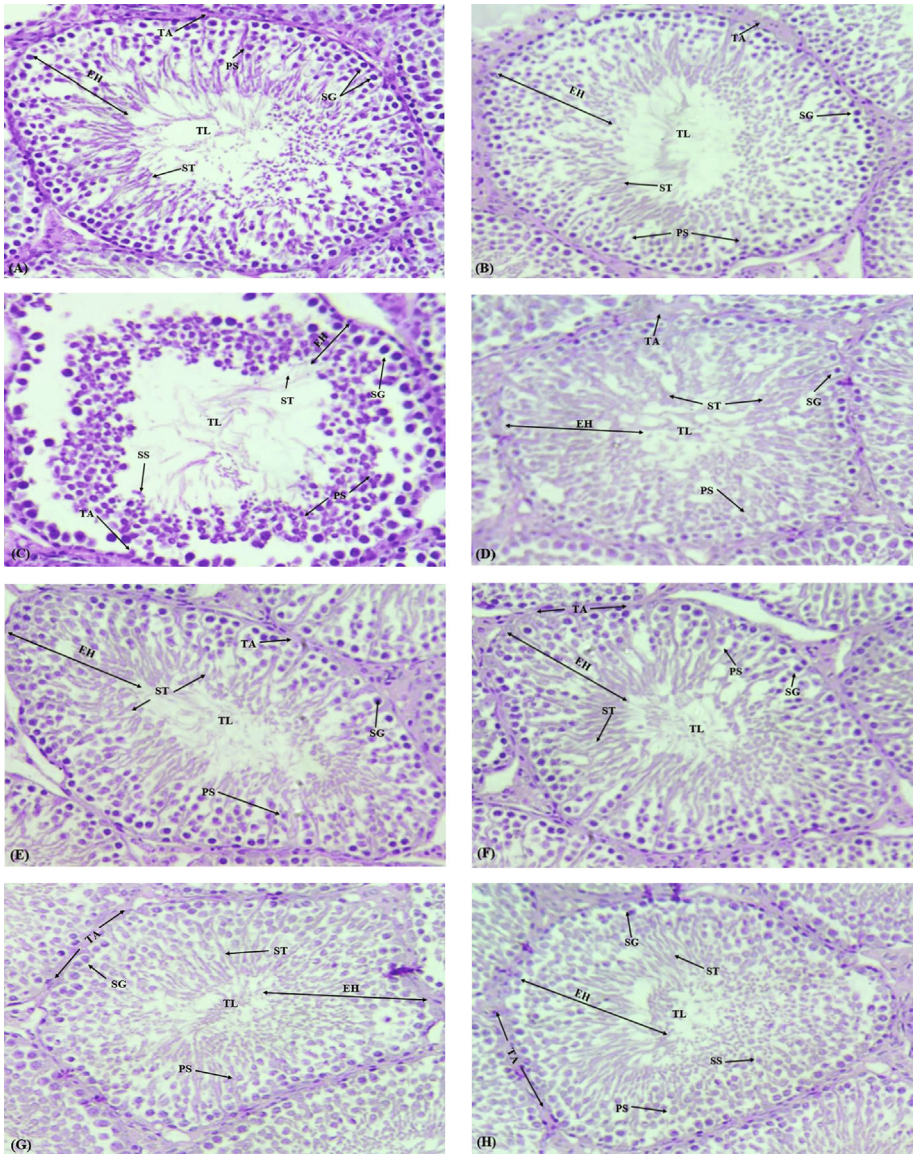

Fig. 1. Histopathological photographs of the control and treated groups from A) Control group exhibiting the dense and healthy germinal epithelium and the slight luminal area comprising spermatozoa. B) Vehicle-control. C) CCl_4 administered group showing empty lumen and deterioration of epithelial layer and interstitial-spaces D) CCl_4 + Silymarin group exhibiting recovery in ST E) CCl_4 + *A. brevifolia* (150 mg/kg) group showing recovered the tubular-lumen loaded with spermatids, germinal epithelium, and reestablishment of collapsed interstitial spaces. F) CCl_4 + *A. brevifolia* (300 mg/kg). More recovery than group E. G) Shows *A. brevifolia* (150 mg/kg) group. H) shows *A. brevifolia* (300 mg/kg) group exposing dense STs with fewer interstitial spaces and standard spermatogenesis with the packed lumen. Epithelial-height (EH), Tubular lumen (TL), Spermatids (ST), Primary-spermatocytes (PS), Tunica Propria (TP), Secondary-spermatocytes (SS), Spermatogonia (SG).

Table 4
Effect of *A. brevifolia* on spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids.

Groups	Spermatogonia (n)	Primary spermatocytes (n)	Secondary spermatocytes (n)	Spermatids (n)
Control	39.00 ± 1.15^{b}	33.33 ± 2.02^{a}	30.66 ± 1.76^{a}	41.66 ± 0.88^{a}
Vehicle control	36.66 ± 3.53^{c}	31.33 ± 2.73^{ac}	28.33 ± 3.48^{ab}	40.66 ± 2.18^{a}
CCl_4	26.00 ± 1.52^{d}	19.66 ± 2.33^{d}	17.33 ± 1.76^{d}	23.66 ± 0.88^{d}
CCl_4 + Silymarin	34.33 ± 0.33^{c}	31.00 ± 0.57^{ac}	27.66 ± 0.66^{b}	35.66 ± 1.33^{c}
CCl_4 + *A. brevifolia* (150 mg/kg)	31.66 ± 0.33^{cd}	29.00 ± 1.52^{c}	24.66 ± 0.88^{bd}	34.66 ± 0.33^{c}
CCl_4 + *A. brevifolia* (300 mg/kg)	34.33 ± 0.33^{c}	30.66 ± 0.66^{ac}	28.33 ± 0.33^{ab}	36.00 ± 1.00^{c}
A. brevifolia (150 mg/kg)	41.00 ± 1.00^{b}	35.33 ± 0.33^{ab}	32.33 ± 1.76^{ac}	42.66 ± 1.45^{a}
A. brevifolia (300 mg/kg)	44.66 ± 0.33^{a}	39.00 ± 1.73^{b}	36.33 ± 1.45^{c}	47.00 ± 1.52^{b}

Values having dissimilar superscripts are significantly ($p < 0.05$) different.

significantly ($p < 0.05$) improved numbers in both the CCl_4 + *A. brevifolia* co-treated groups. Similar was the case in the only *A. brevifolia* treated group, as compared to CCl_4 administrated group as displayed in Fig. 1(A-H) and Table 4. Interstitial spaces and Tubular lumen showed considerable enlargement while the tunica-propria, seminiferous tubules diameter, and epithelial height were significantly ($p < 0.05$) decreased in the CCl_4-treated group as compared to the control group (Table 5). We observed a noticeable restora-

Table 5
Effect of *A. brevifolia* on interstitial spaces, tunica propria, diameter of seminiferous-tubules, epithelial height and tubular lumen.

Groups	Interstitial spaces (μm)	Tunica propria (μm)	ST diameter (μm)	ST epithelial height (μm)	Tubular lumen (μm)
Control	7.62 ± 0.39 [a]	22.89 ± 0.45[a]	178.52 ± 0.59[a]	72.79 ± 0.57[a]	9.74 ± 0.22[a]
Vehicle control	6.76 ± 0.23[b]	21.28 ± 0.04[a]	176.65 ± 0.39[a]	72.13 ± 0.54[a]	9.55 ± 0.33[a]
CCl_4	11.3 ± 0.70[d]	12.31 ± 0.76[d]	166.55 ± 0.99[d]	20.06 ± 0.42[d]	60.9 ± 0.77[d]
CCl_4 + Silymarin	9.25 ± 0.23[c]	17.52 ± 0.76[ab]	177.67 ± 0.16[a]	51.4 ± 0.52[c]	17.3 ± 0.63[c]
CCl_4 + *A. brevifolia* (150 mg/kg)	9.86 ± 0.22[c]	15.54 ± 0.34[c]	176.03 ± 0.38[a]	49.36 ± 0.99[c]	21.2 ± 1.29[c]
CCl_4 + *A. brevifolia* (300 mg/kg)	7.84 ± 0.07[a]	18.57 ± 0.3[ac]	178.28 ± 0.59[a]	52.68 ± 0.47[c]	17.0 ± 1.32[c]
A. brevifolia (150 mg/kg)	7.75 ± 0.29[a]	26.63 ± 0.36[b]	180.46 ± 0.58[b]	74.54 ± 0.31[ab]	12.0 ± 0.96[a]
A. brevifolia (300 mg/kg)	7.45 ± 0.40[a]	27.46 ± 0.89[b]	183.12 ± 0.34[c]	77.08 ± 0.10[b]	12.6 ± 0.54[a]

Values having dissimilar superscripts are significantly ($p < 0.05$) different.

tion in the *A. brevifolia* + CCl_4 (co-treated) histological parameters and the only *A. brevifolia* treated groups compared with the CCl_4 treated group as indicated in Fig. 1 and Table 5.

4. Discussion

The mammalian cells have developed different non-enzymatic and enzymatic processes that could be managed through free radicals. The antioxidant defense system becomes deficient in response to reactive oxygen species (ROS) during oxidative stress situations (Halliwell and Gutteridge, 2007; Ijaz et al., 2020b). The CAT, POD, SOD, and GR activities decrease due to increased ROS production (Abdel-Moneim et al., 2011). The antioxidants generally proceed in a systematic pathway in a bid to cope with oxidative stress-mediated damages. The present study showed that CCl_4 decreased the CAT, POD, SOD, and GR enzyme activities and elevated the TBARS level. Surplus level of H_2O_2 in testes due to CCl_4 instigated the tissue damage and suppressed the antioxidant enzymes (Ojo et al., 2016). The *A. brevifolia* extract scavenged the free radicals and restored enzymatic abilities to trim down the oxidative stress by CCl_4. Bioactive compounds in *A. brevifolia* (as in other species of the same genus) could be a primary reason for decreasing free radicals. Our results are consistent with Shah and Khan (2017), who documented the protective role of *Jurenia dolomiaea* on rat testes. The *A. brevifolia* may shield the testes from oxidative injury by decreasing the free radicals, which corroborates low TBARS level and increased antioxidant enzyme activity.

CCl_4, as a potentially harmful compound, decreased the concentration of FSH, LH, testosterone, and DSP. The present study was initiated by keeping in view the previous observations that testicular atrophy, germinal layer degeneration, reduced testosterone levels, and gonadotropins could be caused by CCl_4 intoxication (Khan and Ahmed, 2009). LH encourages Leydig cells to produce testosterone, and on the other hand, FSH promotes spermatogenesis (Jaffat et al., 2014). Therefore, the CCl_4 intoxication in testicular tissues could be attributed to the toxic effect of CCl_4 by decreasing FSH, LH, and testosterone concentration. The CCl_4 could also disturb the suprachiasmatic hypothalamic nucleus (SCN), which retards the release of gonadotrophins from the pituitary gland (Khan et al., 2011). Increased FSH, LH, testosterone, and DSP levels due to *A. brevifolia* extract could be credited to its immediate effect on SCN, pituitary gland, or gonadal status. The extract of *A. brevifolia* can effectively prevent the CCl4 induced effect on hormone and DSP.

The present investigation illustrated an overall escalation of the cholesterol, triglycerides, and LDL levels, whereas HDL levels exhibited a reduction in CCl_4-administered rats. Lipids play a vital role in cell and organ systems' structural and functional stability (Rawi et al., 2012). The higher levels of lipids, such as cholesterol, triglycerides, and LDL, result in a disordered condition called hyperlipidemia, which generates toxicity in testicular tissues (Mehta et al., 2003). Moreover, the excess plasma level of cholesterol and HDL level reduction results in spermatogenic damages and reduced testosterone concentration culminating in male infertility (Ibrahim et al., 2012). The experimental groups administered with *A. brevifolia* + CCl_4 showed an improved lipid profile as compared to CCl_4 treated group.

CCl_4 administration invoked a remarkable decrease in the germ cells at various stages, whereas we observed an improved cell count in the *A. brevifolia* administered groups. Degeneration of germ cells, seminiferous tubules, increased interstitium, and tubular lumen was evident in the CCl_4-administered group. The proposed presence of flavonoids in genus artemisia may help recover the CCl_4 mediated injuries (Sajid et al., 2016). Similar curative effects were reported by the treatment of *Carissa opaca* plant extracts against CCl_4 caused injuries in testes (Sahreen et al., 2013). In conclusion, our study detected that *A. brevifolia* extract effectively protected the male albino rats from the CCl_4 induced testicular toxicity by restoring the testicular histological alterations. The use of *A. brevifolia* substantially improved the germinal epithelial height, augmented the tunica propria thickness, and increased the diameter of seminiferous tubules.

5. Conclusion

In conclusion, our findings propose that *A. brevifolia* has illustrated curative properties to counter the disturbances in antioxidant enzyme activity and hormonal dysregulation, impairments in the architecture of seminiferous tubules. *A. brevifolia* entails antioxidant and androgenic properties to deal with the sterility issues in animals and humans, instigated by oxidative stress. The protective effects of *A. brevifolia* plant extract on testes may be attributed to its antioxidant and androgenic nature.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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