Research Square

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EFFECT OF METHANOLIC EXTRACTS OF *Artemisia vulgaris* AND *Bergenia ciliata* AGAINST DIETHYL-NITROSAMINE (DEN) INDUCED TESTICULAR CHANGES IN MICE

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Research Article

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Abstract

Background

Diethylnitrosamine (DEN) or N-Nitrosodiethylamine (NDEA) is used as a carcinogen in experimental animal model systems. The DEN treatment at a necrogenic dose can cause acute toxicity to various cell types in the adenohypophysis of the pituitary gland and alter serum levels of several hormones that can alter reproduction. *Artemisia vulgaris* and *Bergenia ciliata* are known for their antioxidant and protective effects against various chemicals.

Objective

The present study aims to elucidate the anti-carcinogenic activity of *Artemisia vulgaris* and *Bergenia ciliata* against diethylnitrosamine (DEN) induced changes in the reproductive system of male albino mice.

Methods

Mice (N = 120), maintained at room temperature (25°C) were divided into six groups (each group containing 20 mice) and treated with saline, DEN, *Artemisia vulgaris* extract, *Bergenia ciliata* extract, DEN+*Artemisia vulgaris* extract and DEN+*Bergenia ciliata* extract respectively for eight weeks. At the end of the experiment, the final body weight was measured and mice were dissected. The weight of both testes was measured, the testicular tissue was processed for histology and blood samples were collected for analysis of testosterone. Histological images were analyzed by ImageJ software for measurement of the area covered by seminiferous tubule (%), area of the interstitium (%), seminiferous tubule diameter (μm), and epithelial cell height (μm).

Results

A significant ($P < 0.05$) reduction in body weight was observed in animals treated with DEN and *Artemisia* co-treated group while body weight was not affected ($P > 0.05$) in *Bergenia* co-treated group. Similarly, a low ($P < 0.05$) level of plasma testosterone (0.2–0.3 ng/ml) was observed in DEN and DEN+*Artemisia* extract-treated groups compared to *Artemisia* and *Bergenia* alone, DEN + *Bergenia* groups. Histological evaluation revealed a significant ($P < 0.05$) reduction in the diameter of seminiferous tubules, epithelial cell height, and cell numbers in DEN treated group compared to the control group. These effects were restored ($P < 0.05$) in the group treated with *Bergenia ciliata* extract but were not restored ($P > 0.05$) in the *Artemisia vulgaris* extract co-treated group.

Conclusion

The results of the present study revealed that *Bergenia ciliata* extract is efficient in reversing DEN-induced DEN-induced reproductive toxicity in male mice. Further studies are needed to evaluate the components of *Bergenia Ciliata* extracts useful in treating reproductive organ toxicity.

INTRODUCTION

Diethylnitrosamine (DEN) is a strong hepatocarcinogen that has been used in rodent models to research liver cancer progression. It increases the clonal growth of started hepatocytes and produces oncogenic mutations [1]. One of the key proteins involved in cancer progression is cdk4, and its overexpression is caused by DEN treatment [2]. DEN exposure modifies liver bioenergetics by blocking mitochondrial complexes I and IV and causes histological alterations, including pre-neoplastic lesions [3]. It also impacts oxidative stress, while the liver initially has some ability to offset these effects through enhanced antioxidant enzyme activity [3]. DEN-induced hepatocarcinogenesis in rats mirrors human hepatocellular carcinoma, making it a useful model for liver cancer processes and treatments [1].

Diethyl nitrosamine (DEN) or N-Nitrosodiethylamine (NDEA) is found in a variety of products like beauty care products, agricultural chemicals, medicinal agents [4], processed meat, cheddar, soybeans, smoke, salted and dried fish, whisky and groundwater. There is sufficient evidence to consider DEN among the most critical environmental carcinogens known to induce unrest in the nuclear proteins required in DNA repair/replication [5]. Since DEN does not itself exert carcinogenicity, it needs to be bioactivated by cytochrome P450 (CYP) enzymes in the liver, resulting in DNA adducts (Segments of DNA bound to a cancer-causing chemical) that form reactive electrophiles through an alkylation mechanism. Alkylation of DNA at the *O*6 position of guanine is an important step in the formation of mutations in cancer, primarily due to the tendency of *O*6-methylguanine to pair with thymine during replication, resulting in the conversion of guanine: cytosine to adenine: thymine pairs in DNA. The reactive electrophiles cause oxidative stress, stimulating cytotoxicity, apoptosis, liver proliferation, and necrosis [6;7]. The carcinogenic effect of DEN can be attributed to its oxidative damage to bio-molecules like proteins, lipids, and DNA [8] leading to cellular injury [9; 10]. Research on diethylnitrosamine (DEN) and its

impact on testicular toxicity and possible medications that mitigate it has recently been conducted. It has been demonstrated that DEN causes oxidative stress, interferes with spermatogenesis, and modifies the hormonal balance in male rodents [11;12]. In human and animal bodies a natural defense system exists that neutralizes reactive oxygen species in cells and protects them from damage [13;14]. However, if ROS production is very high, the natural defense system is rendered ineffective against oxidative stress.

Artemisia vulgaris (Mugwort) and *Bergenia ciliata* (Winter begonia) are therapeutic plants that exhibit anti-cancerous, anti-inflammatory, and anti-bacterial properties [15; 16]. *A. vulgaris* has antiparasitic, antispasmodic, and anti-inflammatory properties and bronchodilator activity. Aqueous and methanolic extracts of *A. vulgaris* exhibited inhibitory effects on cell growth and are attributed to the induction of apoptosis, suppression of cell migration, and autophagy [17]. Various species of Artemisia have shown antiproliferative effects on cancer cells. Extract from *A. maritima* reduced cell migration triggered apoptosis, halted the G2/M cell cycle, and hindered lung cancer cell survival [18]. According to Lian et al. (2018), an extract from *A. vulgaris* also showed cytotoxicity against colon cancer cells by causing autophagy, lowering mitochondrial membrane potential, and preventing cell migration. Extraction from the leaves of *A. vulgaris* inhibited the growth and metastasis of lung cancer cells, brought about cell death, and reduced the activity of the Wnt (Complex network of proteins that aids in cellular regulation) signaling pathway [19]. These results indicate that *Artemisia* extracts may influence apoptosis, cell cycle progression, migration, and other cellular processes, which could make them useful anticancer drugs.

B. ciliata possesses anti-tussive and cytoprotective properties and its rhizome works against urolithiasis. *B. ciliata* is a defensive agent against neoplastic activity [20]. Despite some reports of toxic effects, *B. ciliata* has shown hypoglycaemic effects in rats treated with streptozotocin and shows potential in treating a wide variety of illnesses, including kidney problems [21; 22]. A lot of work has been done on the antioxidant activities of plants against carcinomas in the liver, lungs, and breast. Still, not enough studies are available on testicular toxicity and its amelioration using natural antioxidants. To facilitate gaining a comprehensive understanding of its therapeutic potential and safety profile, additional research is required, as it has the potential to serve as a foundation for the development of anti-cancer treatments.

Androgen and androgen receptor (AR) signaling have a crucial role in the progression of prostate, colon, and breast cancer [16]. Androgens act through the AR, a transcription factor that belongs to the nuclear receptor superfamily. The androgen receptor plays physiological and pathological functions by translocating to the nucleus upon binding to androgens, where it binds to specific DNA sequences [23]. The AR complex regulates the pathological expression of genes [24]. As androgen and AR signaling are intimately involved in cancerogenesis, it stands to reason that androgen in combination with DEN might play an important role in testicular toxicity or carcinogenicity [16]. A lot of work has been done on the antioxidant activities of plants against carcinomas in the liver, lungs, and breast, however, not enough studies are available on the anti-cancerous effect of *Bergenia ciliata* on testicular toxicity. It was hypothesized that *A. vulgaris* and *B. ciliata* can rectify the carcinogenic effect of DEN on the testicular toxicity of albino mice. The objective was to evaluate the impact of *B. clliata* and *A. vulgaris* against diethyl-nitrosamine induced changes in testicular weight, testicular tissue, and testosterone level in male albino mice.

MATERIALS AND METHODS

ETHICAL STATEMENT

The study was approved by the ethical committee of PMAS Arid Agriculture University Rawalpindi for the use of animal experimentally.

DRUGS AND CHEMICALS

Diethylnitrosoamine and NaCl have been purchased from Sigma Aldrich St Louis USA) One percent DEN was prepared by adding 99 ml of normal saline (sodium chloride 0.9 percent) and 1 ml of concentrated diethyl-nitrosoamine solution (0.01µg/µl).

EXTRACT PREPARATION

The plant extracts (*Artemesia vulgaris* and *Bergenia ciliata*) were prepared through conventional extraction methods [25]. The crude extracts were filtered through Whatman filter I and concentrated in a vacuum.

EXPERIMENTAL DESIGN

Balb C mice with an average body weight of about 35-40 g were procured from the National Institutes of Health (NIH), Islamabad, and were kept in an animal house having a temperature of 20-23 °C. The mice were divided into 6 groups containing ten mice in each group. The first group served as control and received saline solution (3.5 µl/g) once a week for eight weeks; the second group was given diethyl-nitrosamine (3.5 µl/g) intraperitoneally once a week for eight consecutive weeks, the third and fourth group received plant extracts (extract 1 and extract 2 respectively, 150 mg/kg) once in a week. The fifth and sixth groups were treated with DEN (3.5 µl/g) along with extract 1 (fifth group) and extract 2 (sixth group) at the dose rate of 150 mg/kg once a week for eight consecutive weeks. At the end of the experimental period, the mice were weighed and dissected. The blood samples and testicular tissue were collected for biochemical and histopathological analysis.

BODY WEIGHT AND TESTICULAR WEIGHT

Relative body and organ weight were measured on an electronic balance.

BIOCHEMICAL ANALYSIS

At the time of dissection, blood samples were collected in tubes containing ethylene-diamine tetra acetic acid (EDTA). Blood serum was separated after centrifugation at 3000 rpm for 10 minutes and stored at -20 °C till further analysis. The serum level of testosterone was measured through an ELISA kit (Amgenix Inc, USA).

HISTOPATHOLOGICAL ANALYSIS

Both the testes were collected, weighed separately, and stored in the refrigerator until histological evaluation.

Testicular Parameters:

The testicular tissue was fixed in buffered formalin for one week, impregnated in equal amounts of xylene, and wax, and incubated for 2 h at 65°C. The sections were double stained in hematoxylin and eosin and mounted with DPX. Tissue sections were observed under a light microscope at 20X and 40X using a Leica microscope (Germany) and photomicrographs were taken at 20X and 40X with an automatic photoshoot system (Cannon, Japan). Slides were examined under a microscope furnished with a computerized camera. Morphometry was done utilizing J image software. The area of the seminiferous tubule and epididymis tubules was measured by planimetry. The morphometric analyses were performed on a cellular level, which allowed a better understanding of the structural alterations brought about by the treatments given to the mice.

Statistical Analysis:

The data on body weight, testicular weight, morphometry, and planimetry were analyzed by ANOVA using CRD and are presented as mean±SEM. When the F ratio was found significant, Tucky's test was used to compare treatment means. A probability value of <0.05 was deliberated as significant.

RESULTS

4.1. BODY WEIGHT AND TESTICULAR WEIGHT

The data on initial and final body weight (Mean±SEM) and weight (Mean±SEM) of the right and left testis of experimental mice are presented in Table 4.1. Significant reduction ($P < 0.001$) in final body weight was observed in DEN-treated mice compared to the control. Similarly, the *Artemisia*-alone-treated mice gained a final body weight lower ($P < 0.05$) compared to the control. The final body weights in DEN+*Artemisia* and DEN+*Berginia* treated rats were significantly lower ($P < 0.001$) compared to the control group. The reduction in final body weight was more pronounced in the *Artemisia-treated* group compared to that treated with *Bergenia*. However, no significant difference was noticed in co-treated groups when compared to the DEN alone treated group (Table 4.1).

The DEN treatment resulted in a significant reduction ($P<0.001$) in the weight (g) of the right as well as left testis of treated mice compared to the control. The testicular weight was similar in all other groups (*Artemisia* and *Bergenia* alone, DEN + *Bergenia*) and the control animals, except the DEN + *Artemisia* treated group. The results showed a significantly higher ($P<0.001$) weight of the testis in all treated groups (*Artemisia* and *Bergenia* alone,DEN + *Artemisia,* DEN + *Bergenia* groups) compared to DEN-treated groups. No significant difference was noted in the DEN + *Artemisia* treated group at $P<0.001$ compared to the control group (Table 4.1.).

4.2. MORPHOMETRY OF TESTICULAR TISSUE

The data on testicular morphometry are presented in Table 4.2. The area (%) covered by seminiferous tubule was significantly reduced ($P<0.001$) in the testis of DEN alone treated group, while, the area (%) covered by the interstitium was significantly higher ($P<0.001$) in DEN treated group compared to *Artemisia* alone*, Bergenia* alone DEN + *Bergenia* and DEN + *Artemisia* groups. No significant difference in the area covered by seminiferous tubule and interstitium was observed in plant extracts alone treated groups. However, in the DEN + *Artemisia* treated group area covered by seminiferous tubule and interstitium was significantly low compared to the control group. Compared to DEN alone treated group, the area of the interstitium and seminiferous tubules was not different in the *Artemisia* + DEN but was significantly high ($P<0.001$) in *Artemisia* alone*, Bergenia* alone, and DEN + *Bergenia* groups Table 4.2. Significant reduction in the diameter and epithelial height was noted in the DEN alone treated group compared to the control group. The diameter in the DEN + *Artemisia* treated group was also statistically reduced ($P<0.01$) compared to the control group however, the other groups (*Artemisia* alone*, Bergenia* alone, DEN + *Bergenia* treatment) groups exhibited no significant change compared to the control. In comparison with DEN alone treated groups, all the groups (*Artemisia* alone*, Bergenia* alone, DEN + *Bergenia*) exhibited significantly high diameter ($P<0.001$ and $P<0.05$) except in the DEN + *Artemisi*a treated groups. The study found no significant variations in epithelial height across any of the treatment groups, except the diethylnitrosamine (DEN)-only group. This shows that the epithelial height was considerably lower in the DEN treatment group than in the control group, indicating a substantial effect. The statistical significance was determined

at a probability threshold of P<0.001, underscoring the robustness of the findings. Other treatment groups, which included those that were given plant extracts or combinations with DEN, did not show significant changes in epithelial height compared to the control group. This indicates that, in contrast to the other treatments, DEN had a targeted impact on changing epithelial height (Table 4.2.).

4.3. CELL COUNT IN SEMINIFEROUS TUBULES

The data on the number of cell types in the seminiferous tubules are presented in Table 4.2. The number of spermatogonia, spermatocytes, and spermatids was significantly reduced (P<0.001) in the DEN alone treated group compared to the control group. When compared to the control group, the total number of distinct cell types did not significantly differ in the groups treated with *Artemisia* and *Bergenia* alone. However, compared to the group treated with DEN alone, there was a statistically significant (P<0.001) increase in the quantity of spermatocytes and spermatids in these groups. Cell counts in the group that received both DEN and *Artemisia* treatment were comparable to those in the group that received DEN treatment alone, suggesting that there was no discernible difference. In contrast to the control group, the cell counts in the DEN + *Artemisia* group were significantly different (P<0.001) (Table 4.2.). In the group treated with *Bergenia* in combination with DEN, cell numbers were recovered and were not different compared to the control group however, the cell numbers were significantly higher compared with the DEN alone treated group (P<0.001, <0.05 respectively; Table 4.2.).

4.4. PLASMA TESTOSTERONE

Testosterone concentration was significantly reduced (P<0.001) in DEN alone treated group and DEN + *Artemisia* treated groups. Statistically significant differences were found between the control group and the treatment groups upon examination of testosterone levels. These differences were seen in the groups that received *Artemisia* alone, *Bergenia* alone, and DEN plus *Bergenia*. Different thresholds of statistical significance were established: P<0.05 for the DEN + *Bergenia* group, P<0.01 for the *Bergenia* alone group, and P<0.001 for the Artemisia alone group. These results show that the treatment groups' testosterone levels were lower than the control group's whereas the difference was strong enough to be considered statistically significant, demonstrating that the treatments exerted an effect on the testosterone levels. The study found that the DEN and DEN + *Artemisia* groups carried much lower testosterone levels than the *Bergenia* group. The *Bergenia* group had higher testosterone levels, which shows that it possessed a protective effect. Figure 4.1.

4.5. HISTO-PATHOLOGICAL OBSERVATIONS

Microscopic study of the testicular sections in different groups showed pathophysiological symptoms in the testis. Testis of the control group animals and animals treated with *Artemisia* and *Bergenia* alone showed normal morphology including compactly arranged seminiferous tubules and thick epithelium. The interstitial space of the testis was also thin and the lumen was sperm-filled. In the DEN alone treated group, seminiferous tubules were arranged loose and interstitium was large. The tubular lumen was empty and disorganized epithelial cells were visible in the seminiferous tubules. Sloughing and vacuolation in the epithelium were the prominent changes observed in the seminiferous tubules of the DEN-treated group (Figure 4.2). The observations indicated that the *Artemisia* co-treatment was less effective while co-treatment with *Bergenia* was more effective against the toxic effects of DEN treatment in the testis of mice (Figure 4.2).

Table 4.1: Mean ± SEM initial and final body weight and testis weight in different treatment and control groups of mice.

Sr.No	**Body Weight**		**Testicular Weight**	
Treatments	Initial weight	Final weight	Right Testis	Left Testis
Control	35±0.91	43.8±1.71	0.238±0.01	0.238±0.007
DEN	34.7±1.00	29.4±0.68***	0.17±0.003***	0.238±0.004+++
Artemisia	35.6±0.81	37.4±1.63*++	0.236±0.005+++	0.238±0.004+++
Bergenia	34.3±1.03	38.4±1.63++	0.234±0.004+++	0.234±0.005+++
DEN + *Artemisia*	35.3±0.95	29.4±1.16***	0.182.004***	0.172±0.004***
DEN + *Bergenia*	35.2±0.85	34±1.00***	0.22±0.006+++	0.224±0.005+++

Values are expressed as Mean ± SEM

*, **, *** represents significant difference at probability value P<0.05, 0.01 and 0.001 as compared to control

+, ++, +++ represents significant difference at probability value P<0.05, 0.01 and 0.001 as compared to DEN treated groups

Table 4.2: Mean ± SEM area and diameter of seminiferous tubules, interstitium, and numbers of different cells in the seminiferous tubules in different treatment and control groups of mice.

Sr.No	Testis Morphometry				Cell Count in Seminiferous Tubules		
Treatments	%Area covered by seminiferous tubule	%Area of interstitium	Seminiferous tubule Diameter (µm)	Epithelial height (µm)	Spermatogonia	Spermatocytes	Spermatids
Control	81.13±1.99	18.87±0.199	139.9±0.83	59.53±1.94	58.94±1.11	71.08±1.61	245.92±2.02
DEN	63.88±0.56***	36.11±0.56***	118.75±0.59**-	46.42.58***	49.4±1.18***	59.2±2.30***	225.84±2.14***
Artemisia	81.72±0.57+++	18.28±0.57+++	146.5±5.87+++	58.3±1.83*+++	57.8±1.06+++	69.48±1.24+++	243.76±3.03+++
Berginia	83.95±0.93+++	16.04±0.93+++	144.55±7.51+++	56.53±1.59+++	59.64±0.74+++	70.32±1.41+++	244.72±2.75+++
DEN + Artemisia	67.79±0.60***	32.20±0.60***	120.32±0.41**	52.46±1.71+	50.68±1.29***	60.58±1.92***	227.8±2.26***
DEN + Berginia	76.90±0.46+++	23.09±0.46+++	136.65±0.94+	56.1±0.40+++	55.96±1.26+++	67.18±1.24+	236.84±2.23+

Values are expressed as Mean ± SEM

*, **, *** represents significant difference at probability value P<0.05, 0.01 and 0.001 as compared to control.

+, ++, +++ represent significant differences at probability value P<0.05, 0.01, and 0.001 as compared to DEN treated group.

DISCUSSION

Diethylenitrosamine (DEN), the potent environmental carcinogen has been known to induce damage in many enzymes involved in DNA repair/replication and is specially used to cause hepatocellular carcinoma in experimental animal models [4]. Rhodamine dyes, which can produce nitrite-rich water in concentrations as high as 75%, are one of the primary environmental sources of DEN [26]. Exposure to treated water could potentially endanger the health of populations. At least 18 animal species, including poultry and cats, have identified DEN as carcinogenic, indicating its potential as a human carcinogen [27]. The pervasive carcinogenic potential of DEN underscores the significance of understanding its sources and mechanisms of action for public health protection.

The DEN bioactivation in rat liver involves α-hydroxylation of DEN by CYP2E1 and other P450 isozymes leading to the reactive ethyl diazonium ion [28]. It has also been reported that there is a close association between guanine O-alkylation and mutation frequency [29]. According to Tong (1982) [30], O6-chloroethyl guanine may crosslink with the opposite cytosine residues, thereby blocking DNA replication. DEN exposure of cells disturbs the balance between antioxidants and reactive oxygen species and results in carcinogenesis [3]. In the present study, a significant reduction in body weight was observed in mice that got DEN treatment for eight weeks. Reduction in body weight can be attributed to collective tissue damage in the body by initial toxic lesions in liver cells, and reproductive cells, as well as due to reduction of food intake [31].

Plants of the genus *Artemisia* have revealed cytotoxic effects against cancer cells by inducing cell cycle arrest [32]. The anticancerous activity of *Artemisia* species has been observed against human cancer cell lines especially A549, HeLa, and MCF7 [33]. *Artemisia* has bioactive compounds like alkaloids, terpenoids, flavonoids, and coumarines and possesses potential antioxidant activity against aging, inflammation, and chronic infectious diseases. However, in the present study, *Artemisia* did not ameliorate the damaging effects of DEN on body weight in DEN-treated mice. It might be possible the “concentration of extract” of *A. vulgaris* that was used during the experiment acted as a pro-oxidant and lost its efficiency against the potent diethylnitrosamine.

Bergenia ciliata is a perennial herb having antibacterial, antioxidant, anti-inflammatory, anti-diabetics, and antiviral activities [34]. *Bergenia* treatment was efficient in maintaining a normal body weight. However, the beneficial effect of *Bergenia* in terms of body weight might be attributed to its anti-oxidant activity [35]. There is substantial therapeutic potential in Bergenia species, as evidenced by numerous studies. *Bergenia ciliata* demonstrated prophylactic effects against nephrolithiasis in rats, which were presumably mediated by its antioxidant activity [36]. Likewise, bergenin, which was isolated from *Bergenia ligulata*, exhibited potent antioxidant and antilithiatic properties, effectively shielding against oxidative stress and renal dysfunction in hyperoxaluric rodents [37]. Our results are in line with those of the previous study that reported the projected effects of *Bergenia* against tissue/organ/animal body weight loss due to exposure to hazardous chemicals [38].

It was observed that DEN treatment induces degenerative changes in the testicular tissues of mice. These changes were successfully ameliorated by *Bergenia* while *Artemisia* exhibited less or no protective effect against DEN-induced reproductive toxicity the reason may be the same as above. The structural and functional integrity of testicular tissues depends on the circulating androgens and a small decrease in androgen content can result in a reduction in the weight of the testes [39]. In the present study, testicular weight was significantly reduced in the DEN-treated group as has previously been reported by [40]. The mice treated with *Bergenia ciliata* extract restored normal testicular weight compared to DEN treated group

because it is an excellent antioxidant that has the potential to directly scavenge or prevent the generation of ROS [35]. It is relevant to mention that *Bergenia* protects tissue from tissue weight loss [41]. However, the effect of *Artemisia* was not pronounced which is contradictory to previous literature [42].

The structural and functional integrity of testicular tissues depends on the circulating androgens and a small decrease in androgen content can result in a reduction in the weight of the testes [39]. In the present study, testicular weight was significantly reduced in the DEN-treated group as has previously been reported by [40]. The mice treated with *Bergenia ciliata* extract restored normal testicular weight compared to DEN treated which can be attributed to the antioxidant activity of *Bergenia* to directly scavenge or prevent the generation of ROS [35] and to protect the tissue from tissue weight loss [41]. The seminiferous tubules function to produce, maintain, and store sperm. The reduction of the seminiferous tubule width in mice shows that the shriveling of these tubules leads to structural disruption in sperm production in the testis [43]. In the present study, the % area covered by seminiferous tubule was significantly reduced in the testis of the DEN alone treated group compared to the control group. The interstitial cells of leydig are responsible for testosterone production. Similarly, the % area covered by the interstitium was significantly higher in DEN treated group than control. The increased % area of interstitium might be due to inflammation. The protection provided by *Bergenia* ciliata against DEN-induced tubular lesions and reduction in epithelial height as well as diameter might be due to the potent antioxidant potential of the plant [35]. However, the results are not in line with Anibogwu (2021)[44] who reported the antioxidant effects of *Artemisia* in embryonic cells. As the DEN acts by inducing oxidative stress resulting in cell death [40], the *Artemisia* however, did not protect the tissue either due to lack of its antioxidant potential or due to certain other mechanisms [44]. *Bergenia* co-treatment resulted in reversing the reduction in the number of cells in the seminiferous tubules but *Artemisia* failed to increase the number of cells. This protective effect of *Bergenia* might be due to its antioxidant potential [35] however, the lack of effect of *Artemisia* in protecting testicular tissue is contradictory to previous findings [44]. Further studies are required to confirm the effects of these plant extracts.

In the target tissues, androgens enter the cell cytoplasm by simple diffusion across the cell membrane. Once inside the cell, the androgens bind and activate the androgen receptors. The androgen-receptor complex attaches to a specific DNA site and stimulates the production of messenger RNA, which, in turn, stimulates the production of the enzymes and proteins necessary to affect androgen action [45]. The level of testosterone was increased in all the low-dose DEN-treated groups except for chronic exposure to DEN. High-dose administration of DEN reduced the testosterone level in the blood [40]. A significant reduction in testosterone might have caused pathological changes in the testis tissue [46]. Plant extracts as well as other chemical agents cause hormonal imbalance as alkaloids and flavonoids reduce plasma concentrations of LH, estradiol, and FSH [47]. In this study Testosterone concentration was significantly reduced in the DEN alone treated group and DEN + *Artemisia* treated groups Diethyle nitrosamine is a potent genotoxin and being an endocrine disrupter might alter the nature of those enzymes that are responsible for androgen production. No significant difference was noted in other treated groups regards to control group.

CONCLUSION

In conclusion, the results of the present study indicate that DEN can induce reproductive toxicity by affecting the HPG axis and ultimately the seminiferous tissue. These effects are reversed by *Bergenia* co-treatment but could not be reversed by *Artemisia* co-treatments. Hence, *Bergenia* is a more potent protective agent than *Artemisia* against DEN-induced reproductive toxicity. Further studies are obligatory to endorse the effects of these extracts against other chemical toxicants.

Declarations

Conflict of Interest

Authors have no conflict of interest to declare.

AUTHORS CONTRIBUTION

S.S., and T.A., were involved in practical work. S.S. and S.J. analyzed the data. S.S. and S.A. drafted the manuscript.

Availability of Data and Material

Additional information will be provided upon request.

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Figures

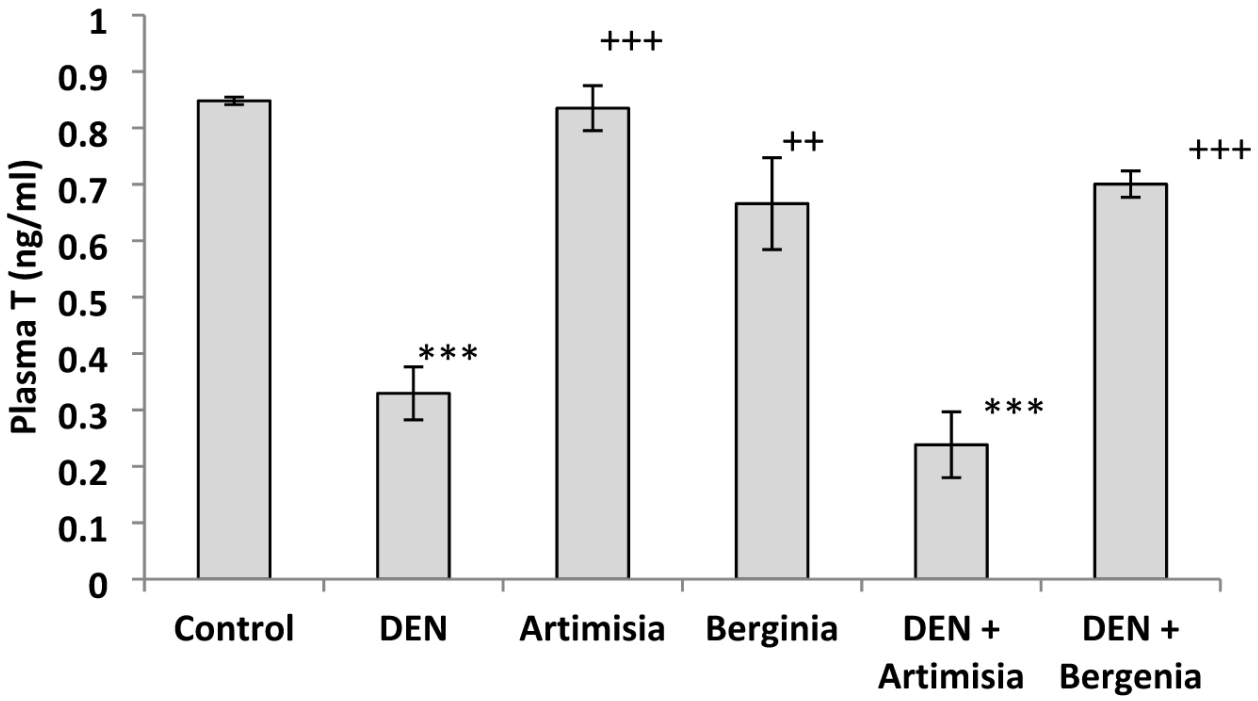

Figure 1

Figure 4.1: Mean ± SEM plasma testosterone concentration (ng/ml) in control and different treatment groups of mice. *, **, *** represents significant difference at probability value P<0.05, 0.01 and 0.001 as compared to control. +, ++, +++ represent significant differences at probability values P<0.05, 0.01, and 0.001 as compared to the DEN-treated group. ANOVA followed by Tukey's test.

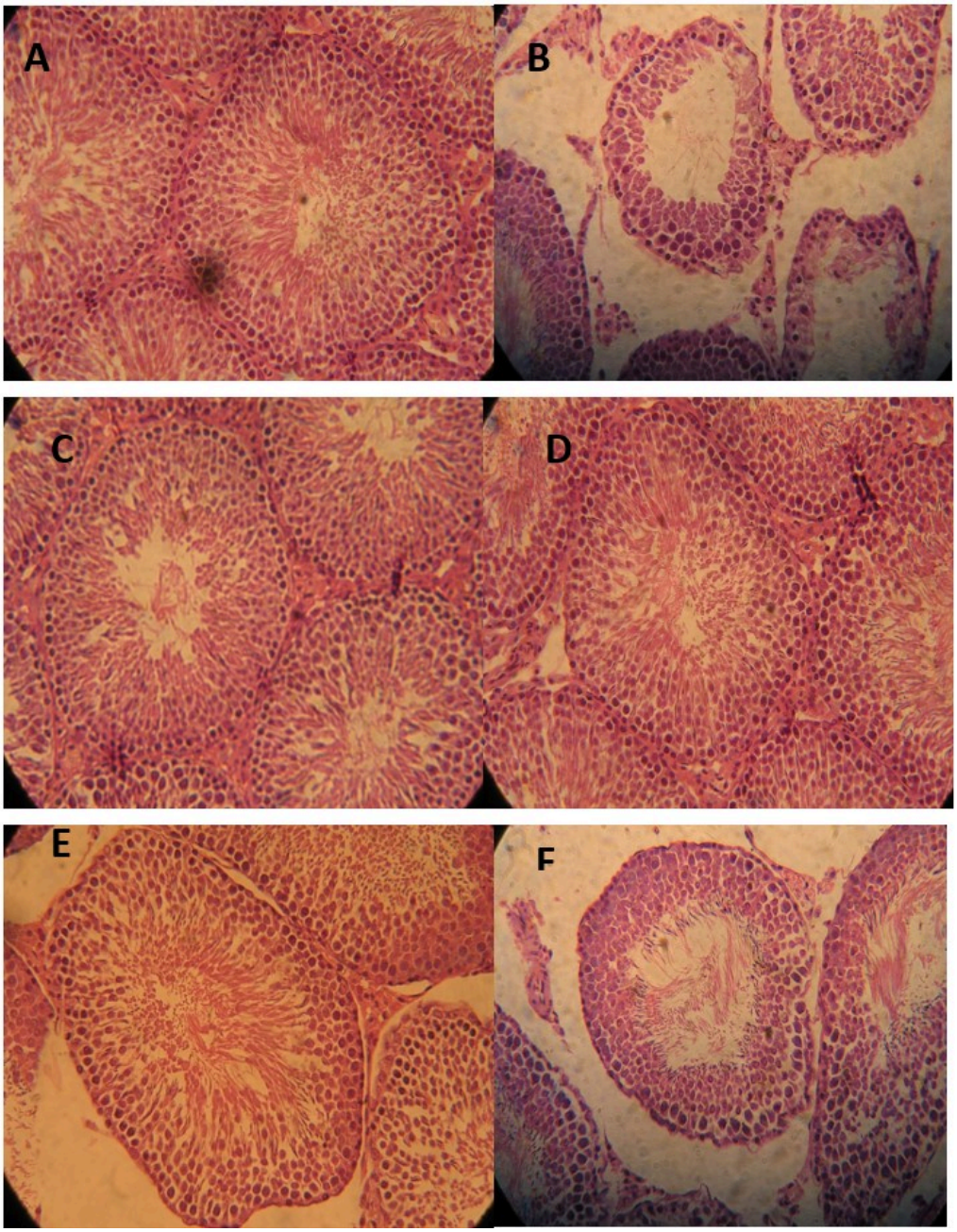

Figure 2

Figure 4.2: Photomicrograph of seminiferous tubules of the control and different treatment groups. A: control testis having closely arranged tubules and sperm-filled lumen. B: DEN treated group with shrunk seminiferous tubules and empty lumen. C, D: seminiferous tubules similar to the control group. E: represents seminiferous tubules with degenerative changes like in the DEN alone treated group. F: represents seminiferous tubules with improved diameter and filled lumen.